

Figure 1: Examples of Nuclease Stable Ribozyme Motifs

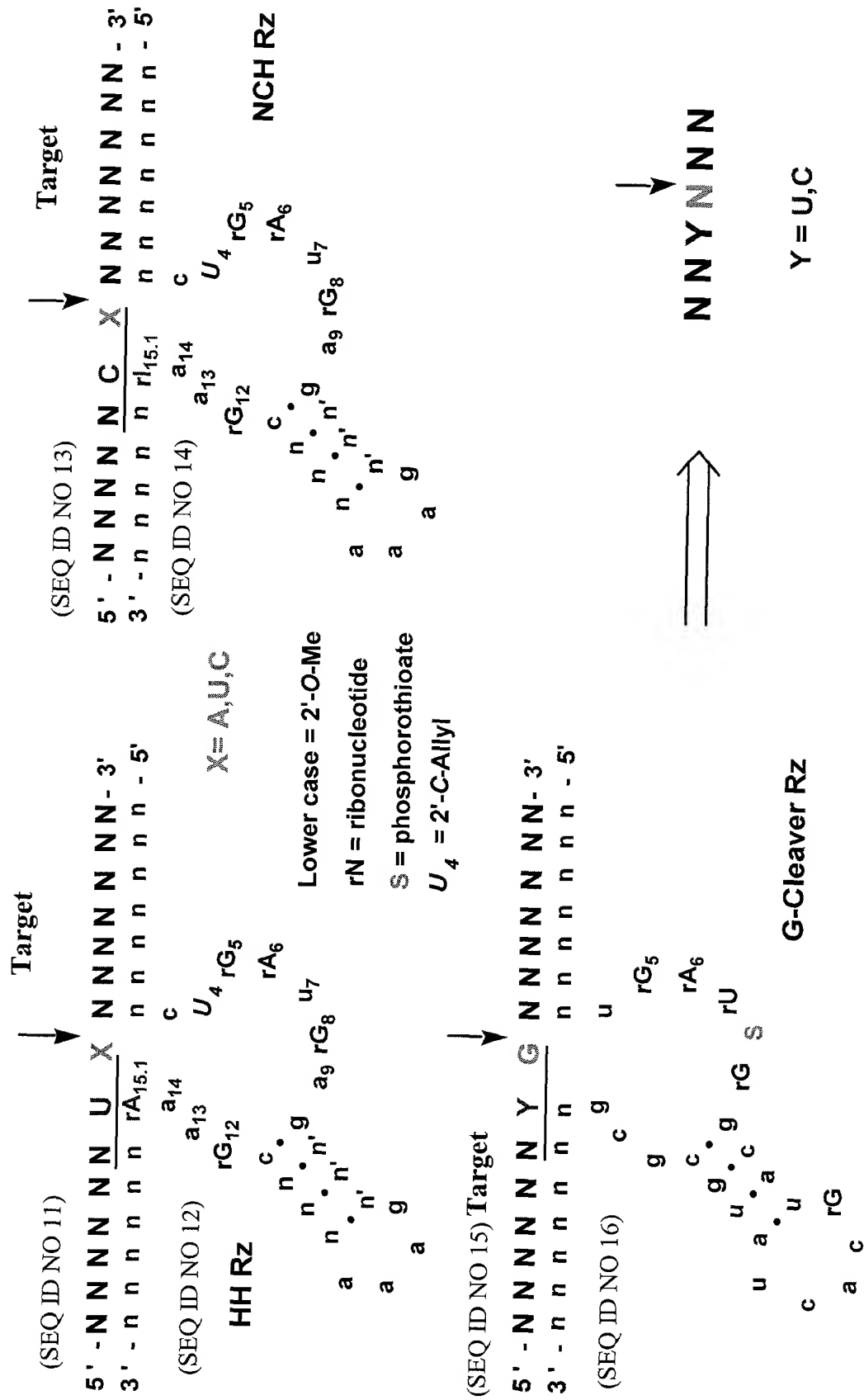


Figure 2: 2'-O-Me substituted Amberzyme Enzymatic Nucleic Acid Motif

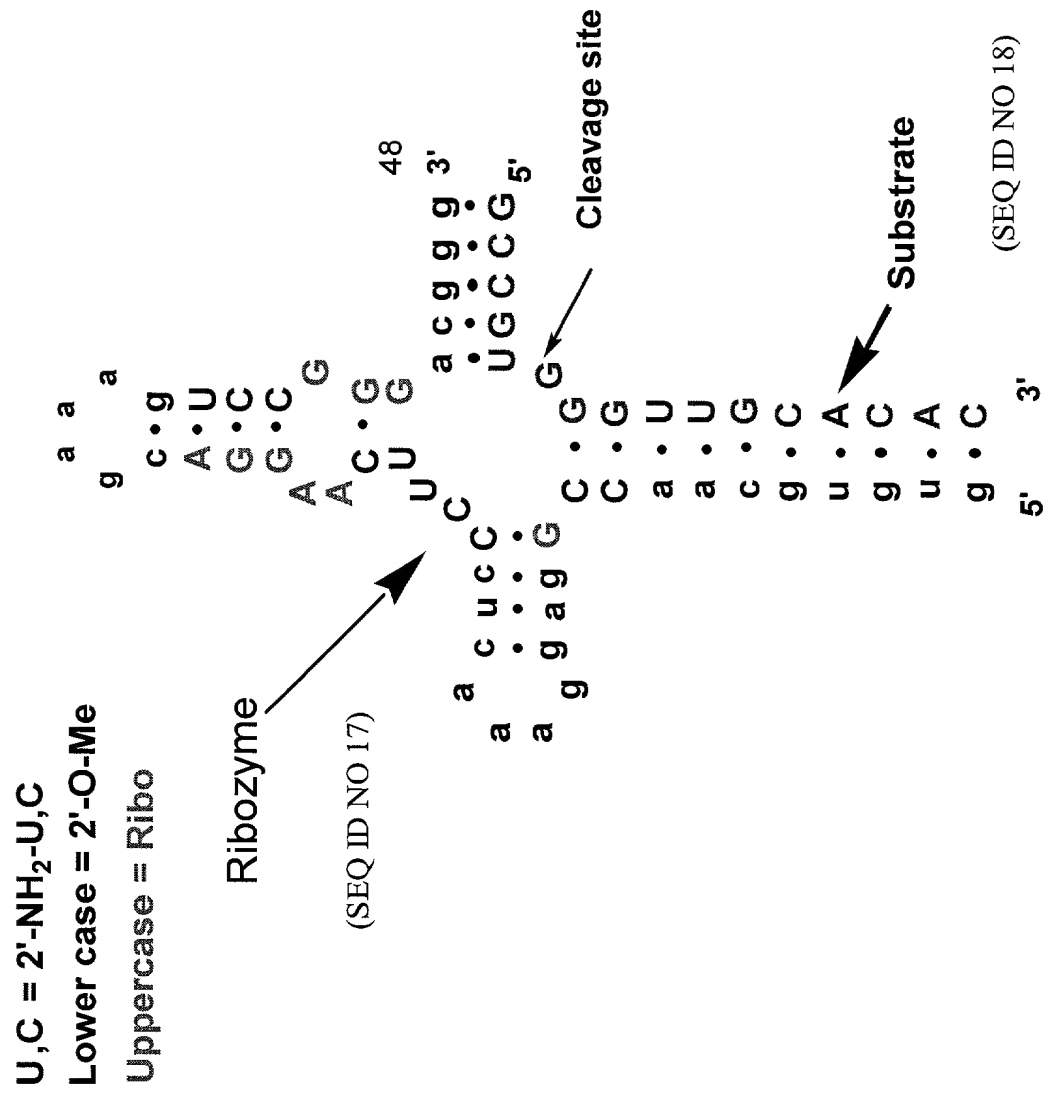
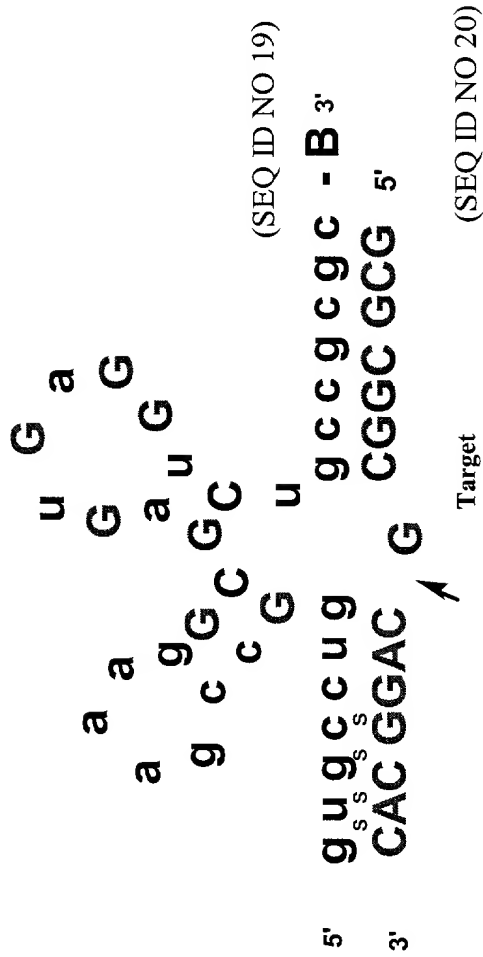


Figure 3: Stabilized Zinzyme Ribozyme Motif

Zinzyme A-motif RZ



Legend

Uppercase indicates natural ribo residues

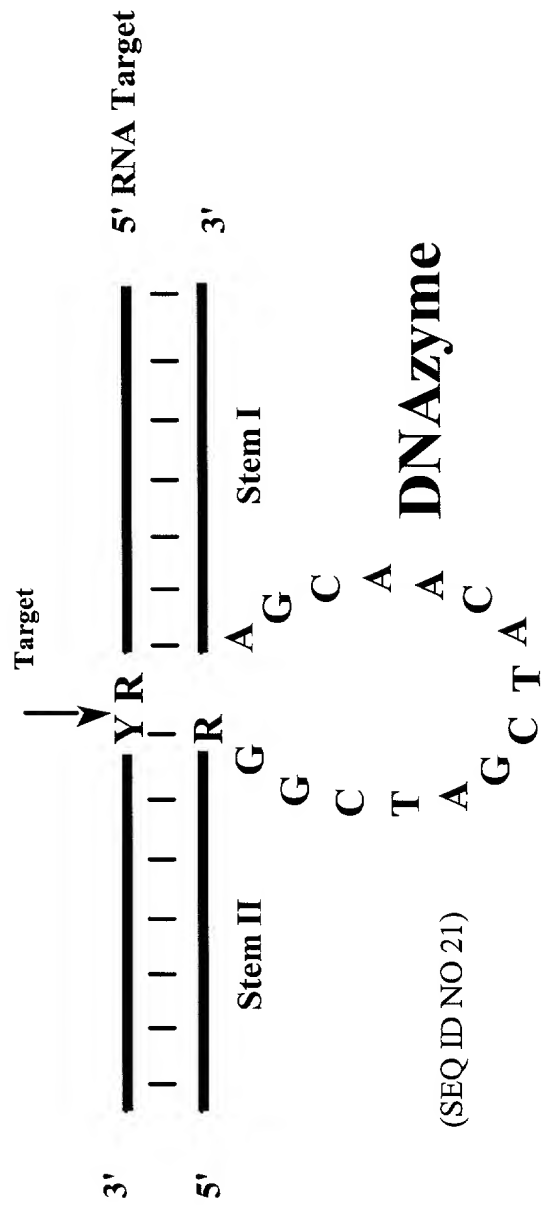
C indicates 2' - d-NH₂-C

Lowercase: 2'-O- Me

Subscript _s indicates phosphothioate linkage

B: 3'-3' abasic moiety

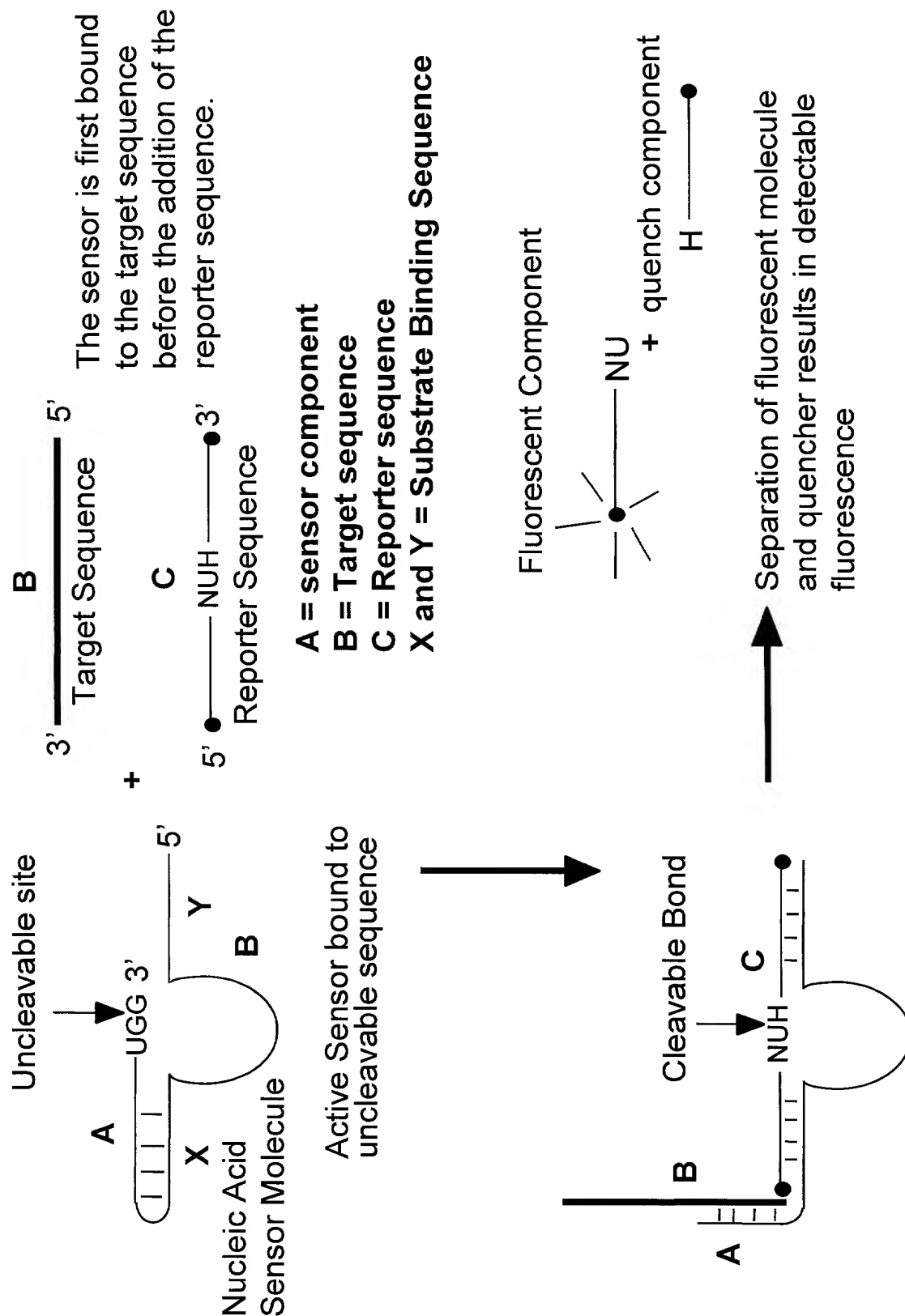
Figure 4: DNAzyme Motif



Legend

Y = U or C
R = A or G

Figure 5. Detection of Target Sequence Using a Cis-Blocking Sequence



A. Bound to the target sequence. Accessible by the reporter oligonucleotide. The enzymatic nucleic acid is unbound from its sensor component allowing for normal catalytic activity.

B. The nucleic acid catalyst binds to the sensor component which prevents the catalytic activity of the molecule. Binding is to either the substrate binding arms or to nucleotides within the sensor molecule core.

Legend:

- Sensor Component
- ▨ Nucleic Acid Catalyst
- Linker (Present or Absent)

Figure 7a. Examples of Diagnostic Effector Molecules

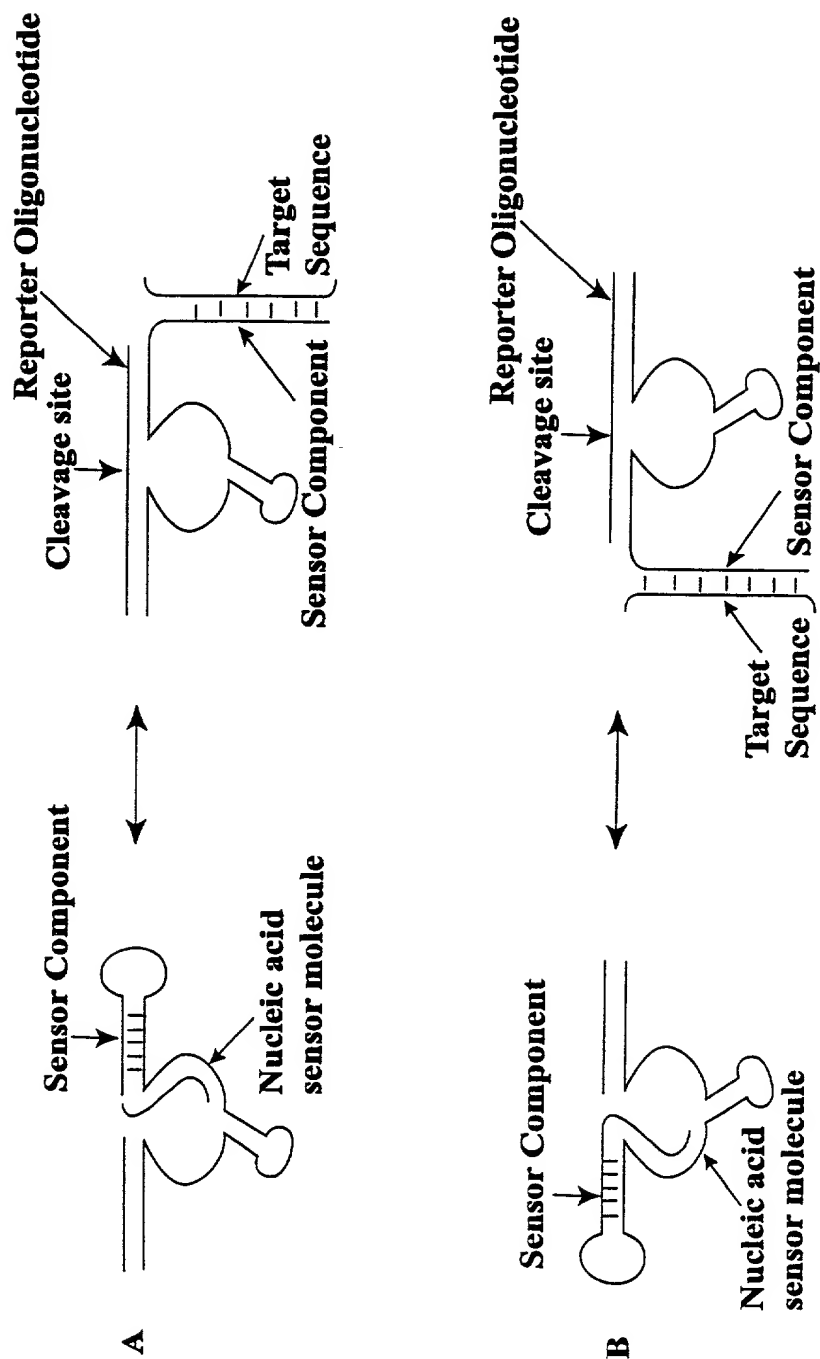


Figure 7b. Examples of Diagnostic Effector Molecules

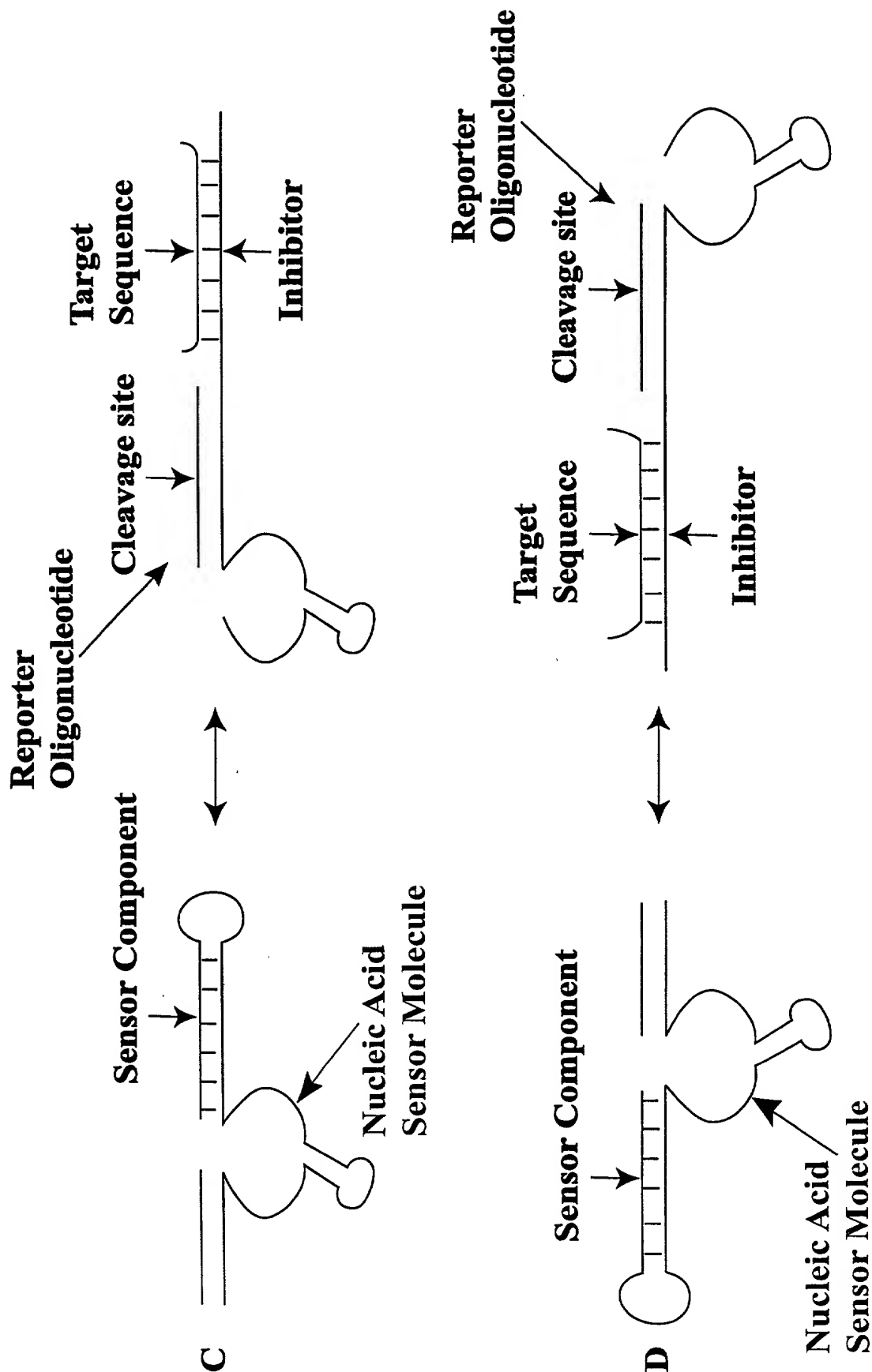


Figure 8a. Examples of Diagnostic Effector Molecules

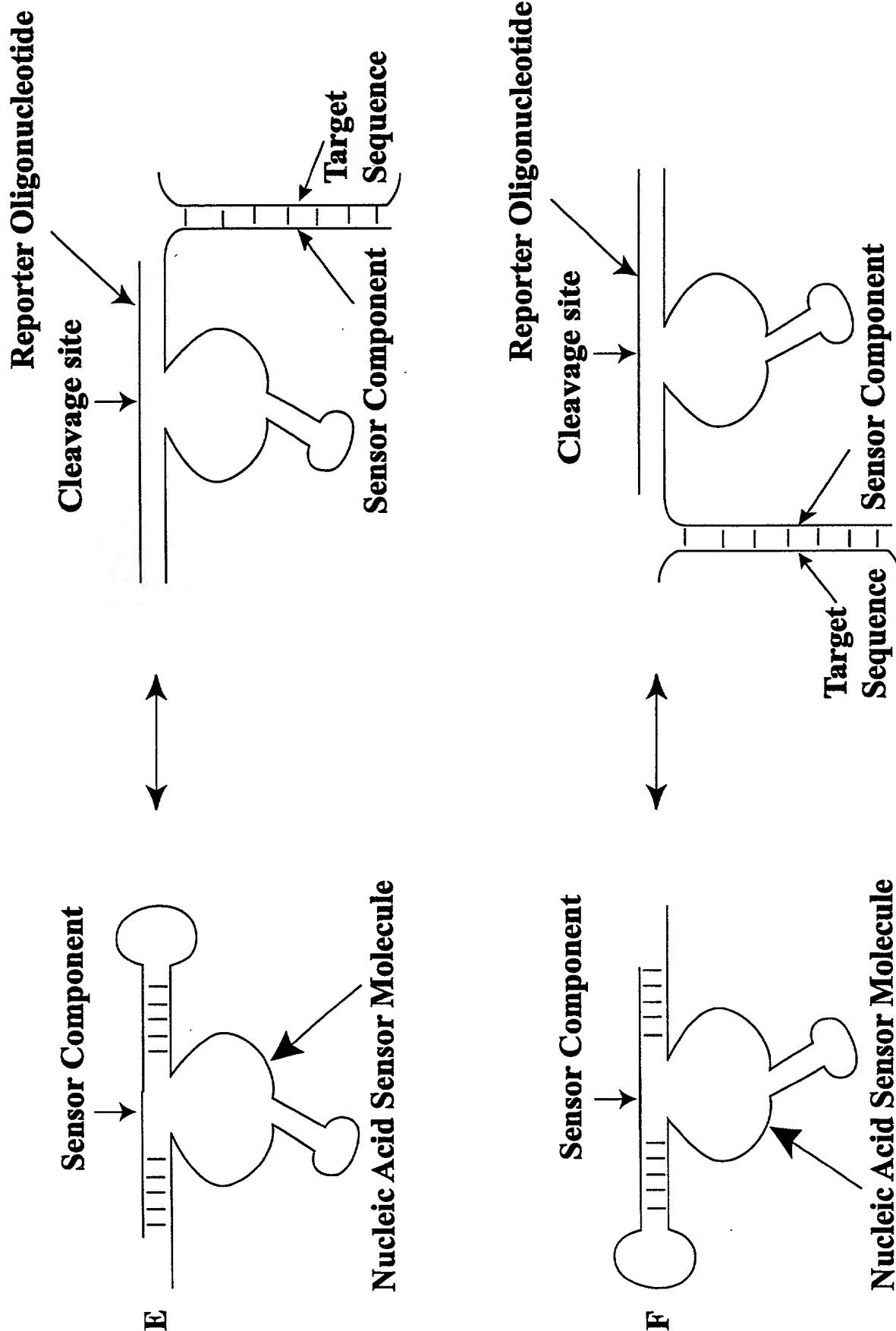


Figure 8b. Examples of Diagnostic Effector Molecules

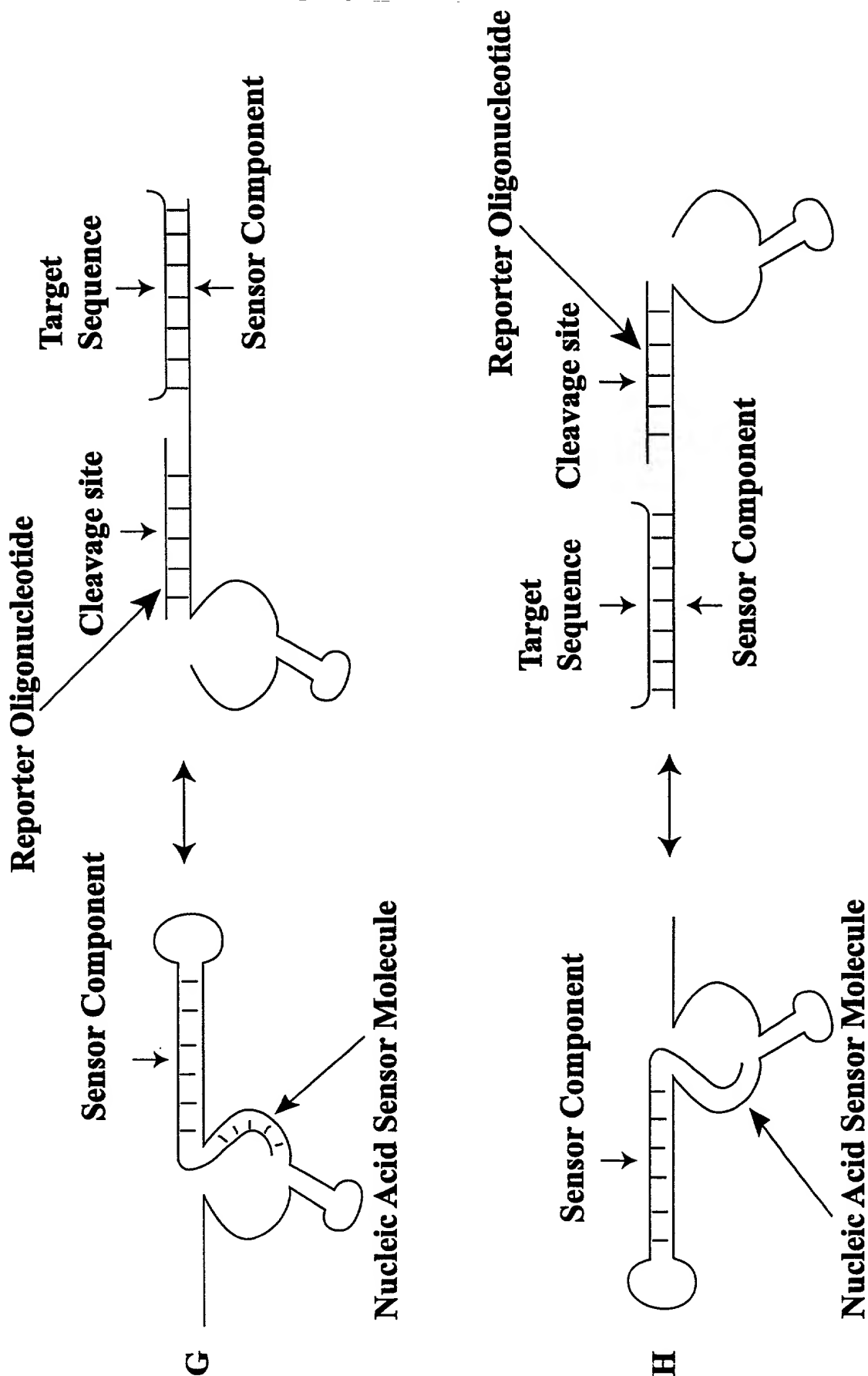


Figure 9. Examples of Diagnostic Effector Molecules

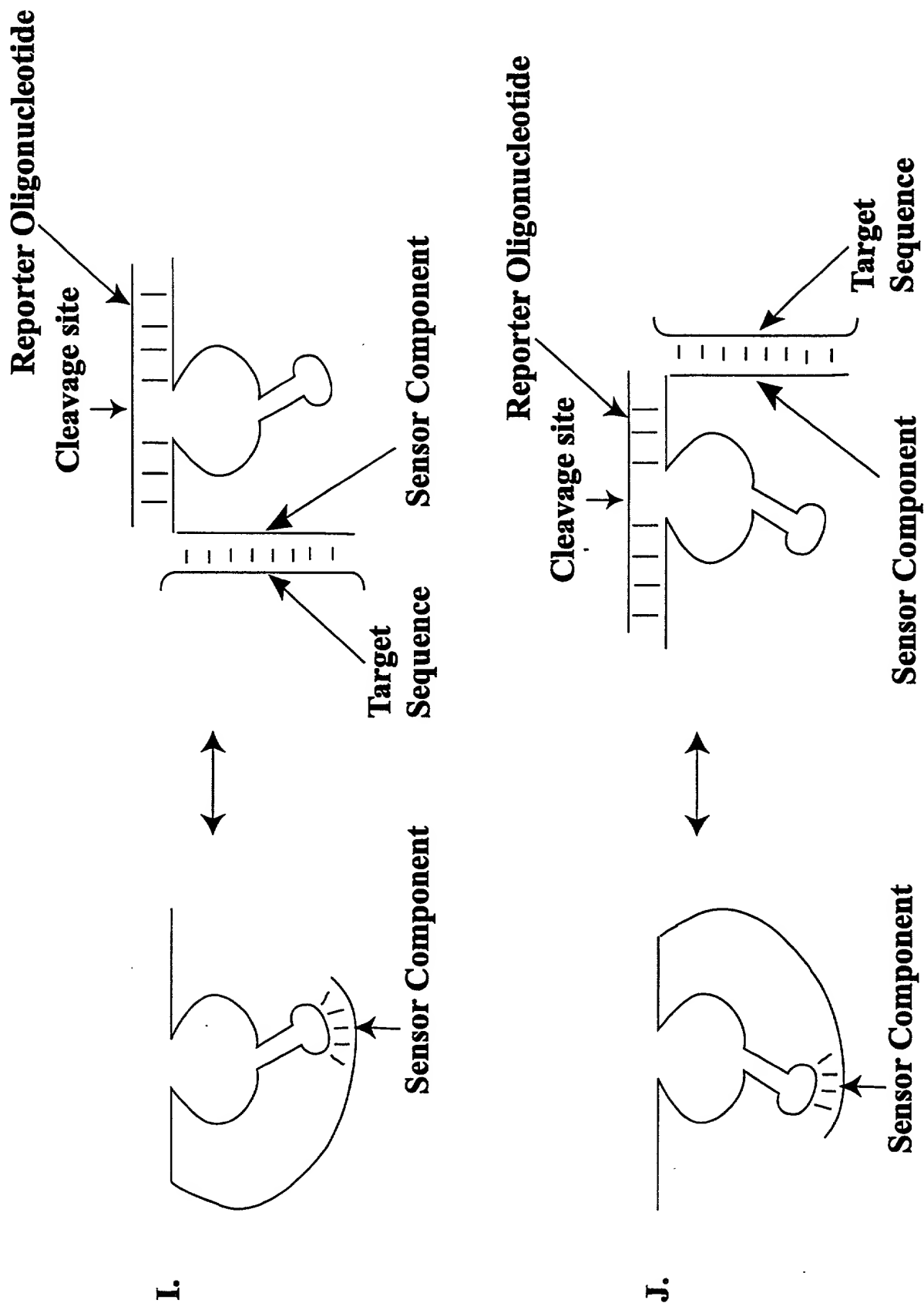


Figure 10: Examples of Diagnostic Effector Molecules

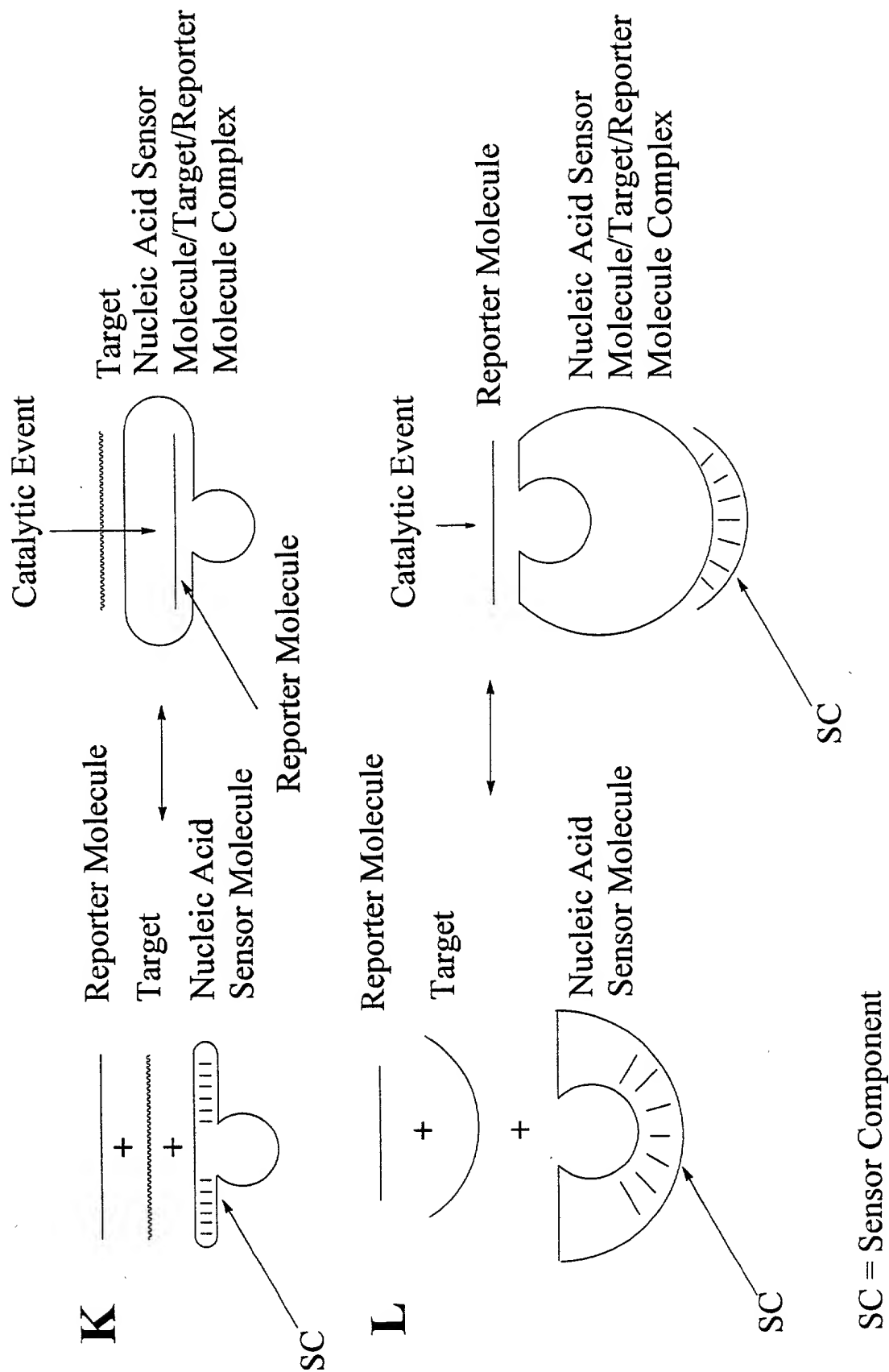
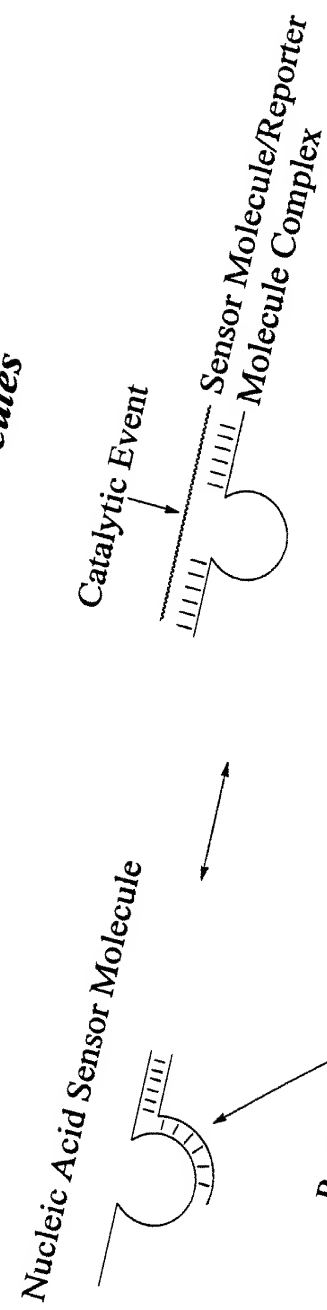


Figure 11: Examples of Diagnostic Effector Molecules

M



N

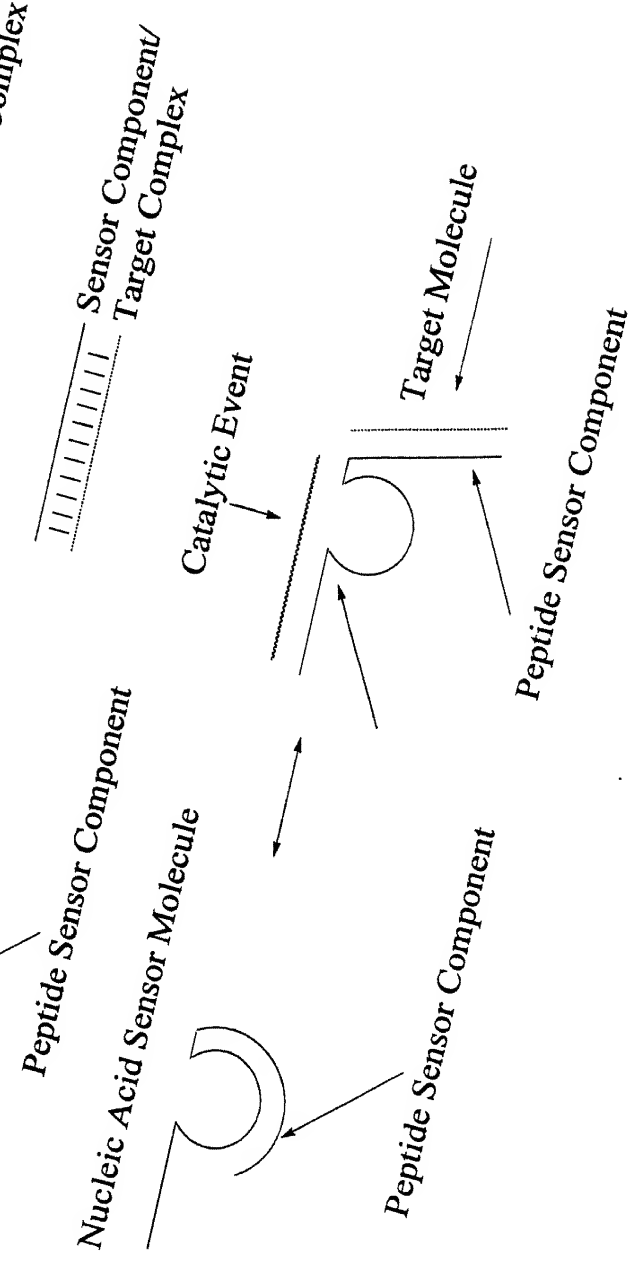
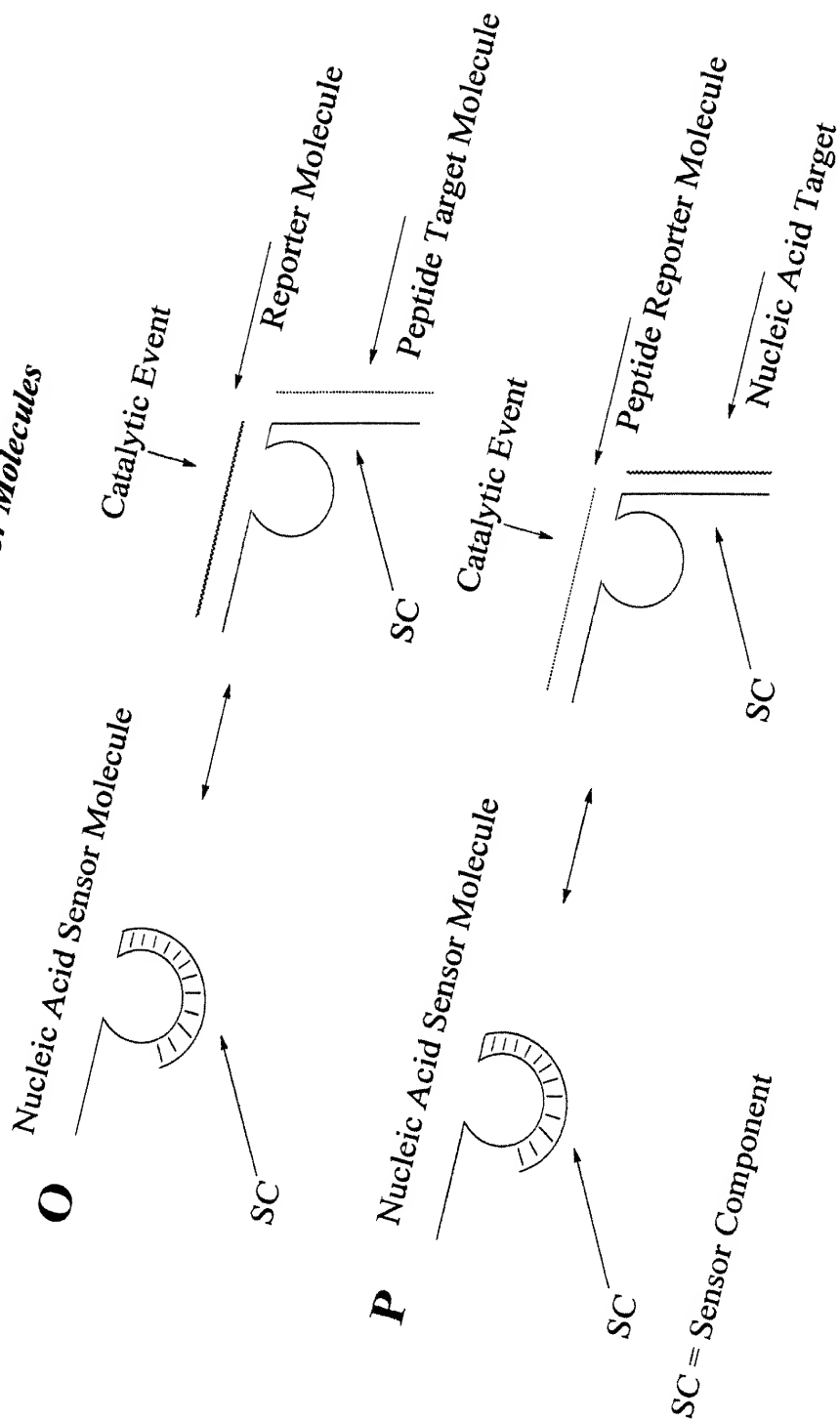


Figure 12: Examples of Diagnostic Effector Molecules



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Figure 13: Examples of Diagnostic Effector Molecules

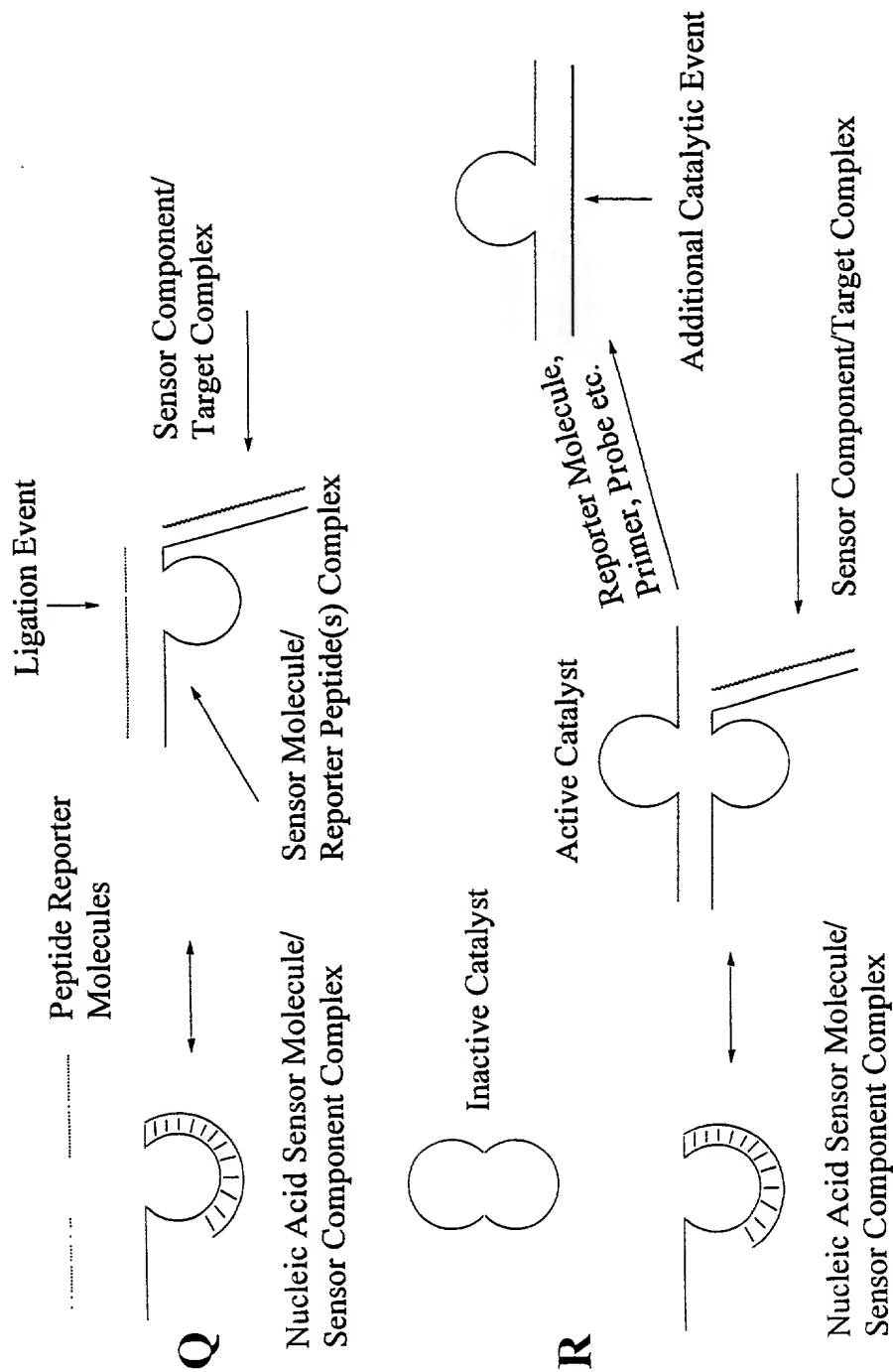


Figure 14: Inherent Amplification of Signal

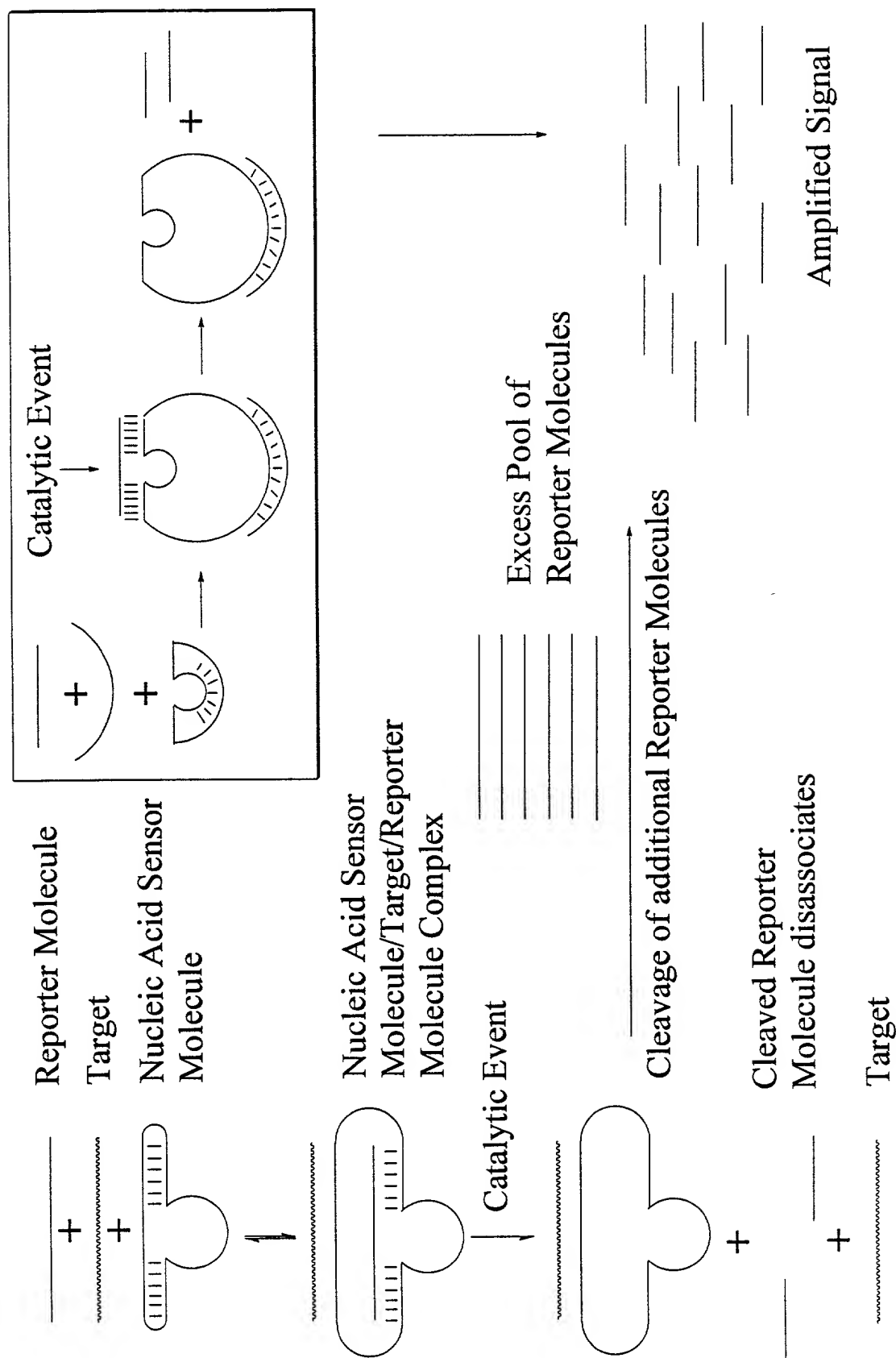


Figure 15: Example of Diagnostic System

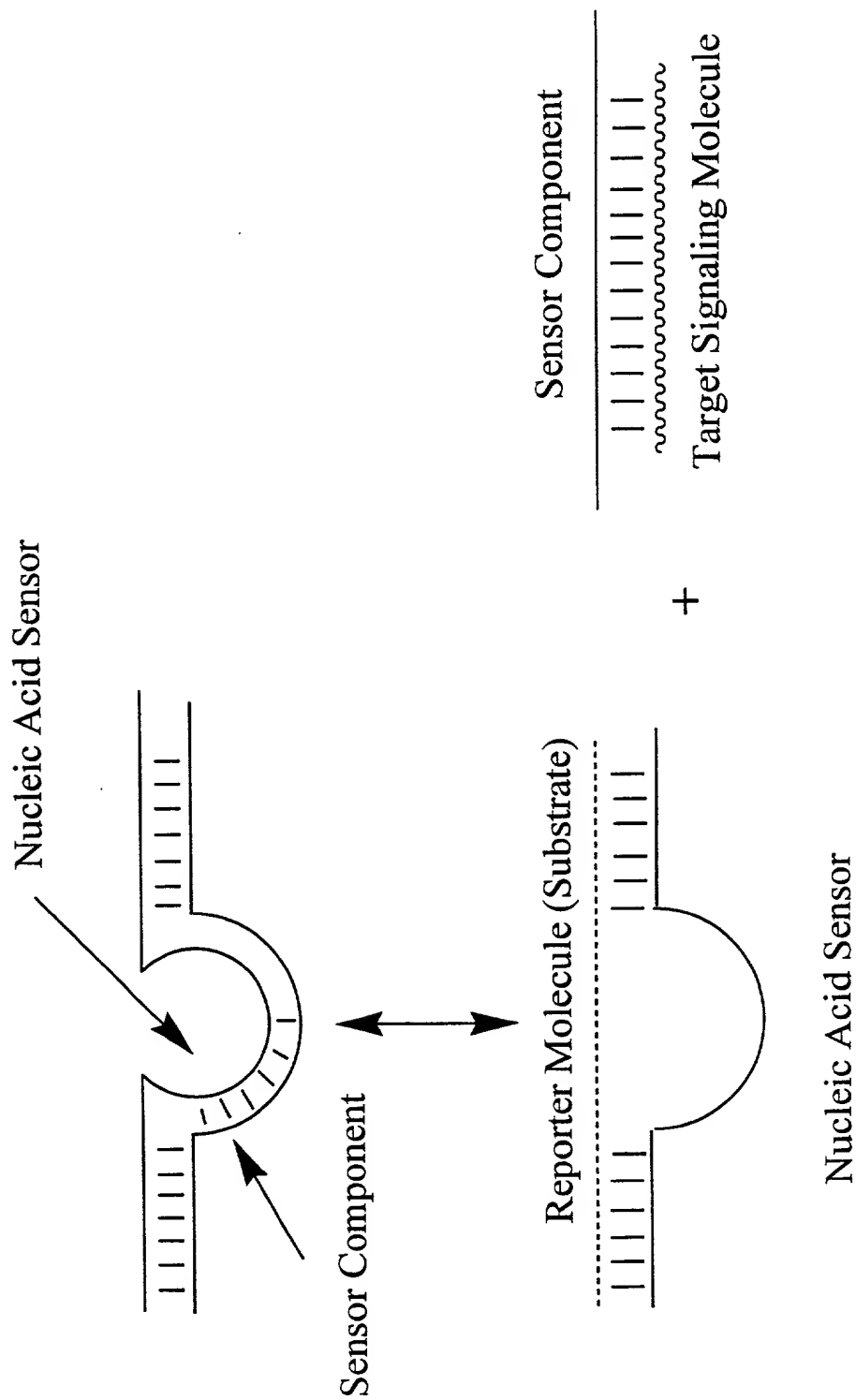


Figure 16: Diagnostic Screen
 Inhibitory Folding with Target Rescue

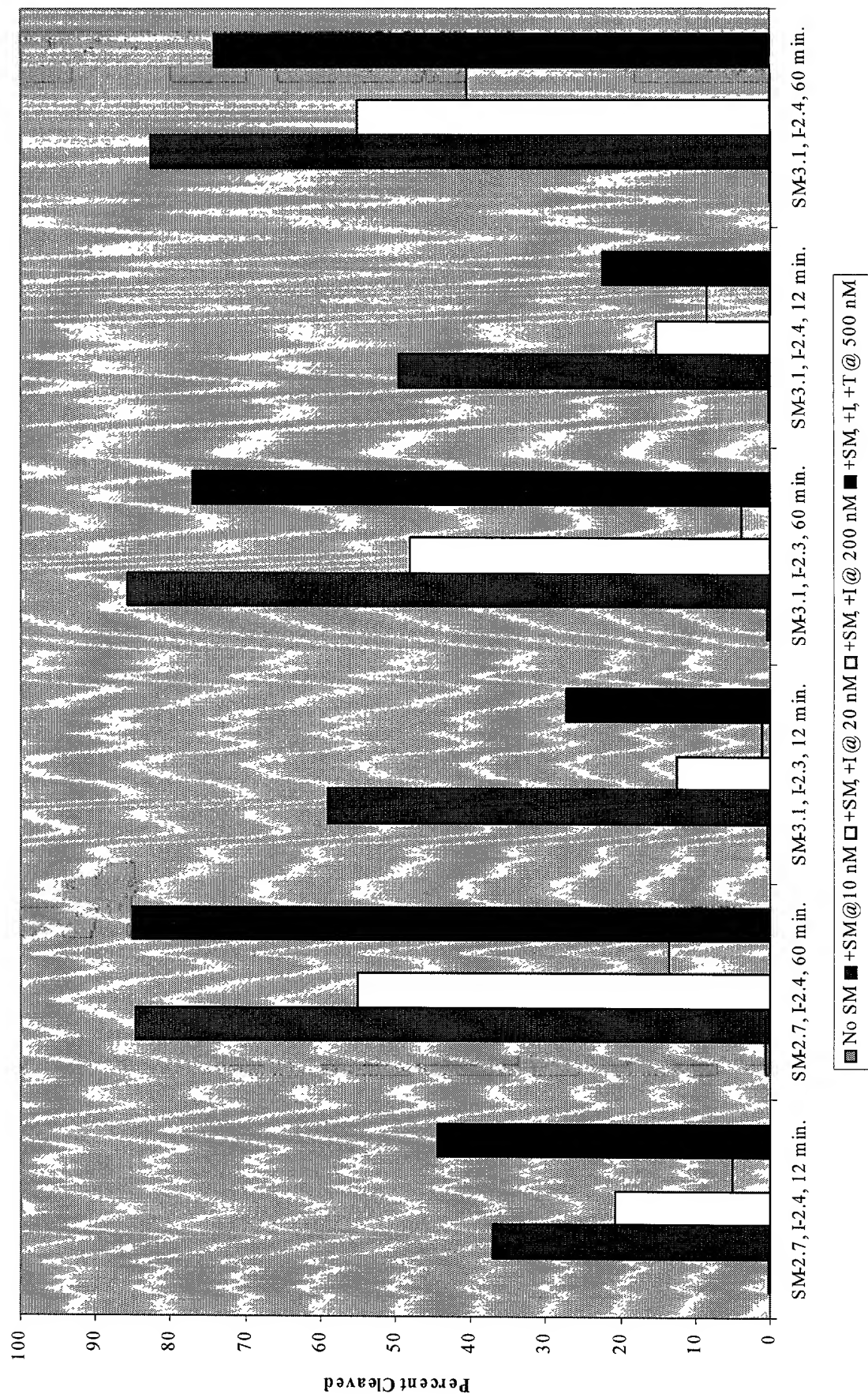


Figure 17a: Auto-ligation Nucleic Acid Sensor Molecules - Selection Scheme

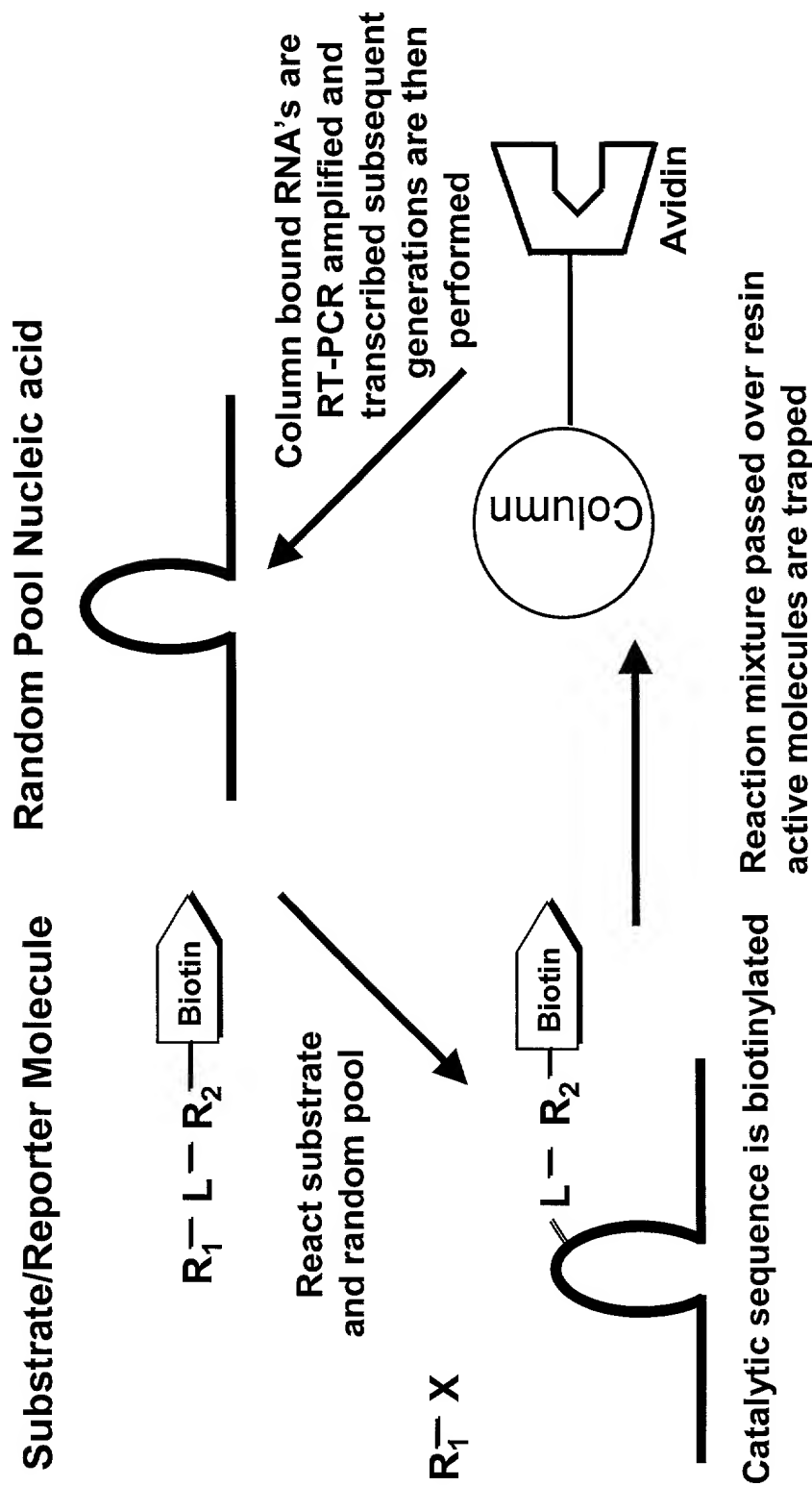
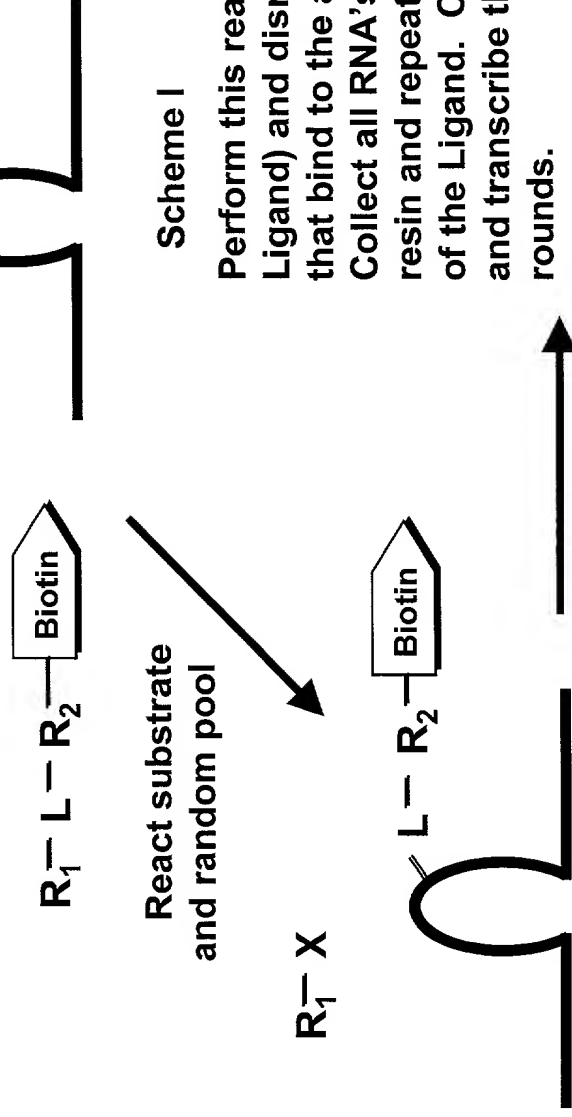


Figure 17b: Auto-ligation Nucleic Acid Sensor Molecules - Ligand Dependent

Substrate/Reporter Molecule + Random Pool Nucleic acid

- Ligand (first round)
- + Ligand (second round)



Scheme 1

Perform this reaction (in the absence of the Ligand) and disregard the molecules that bind to the avidin resin.
Collect all RNA's that flow through the avidin resin and repeat the reaction in the presence of the Ligand. Collect and RT-PCR amplify and transcribe these molecules for subsequent rounds.

Catalytic sequence is biotinylated

Reaction mixture passed over resin
active molecules are trapped

Figure 17c: Auto-ligation Nucleic Acid Sensor Molecules - Ligand dependent

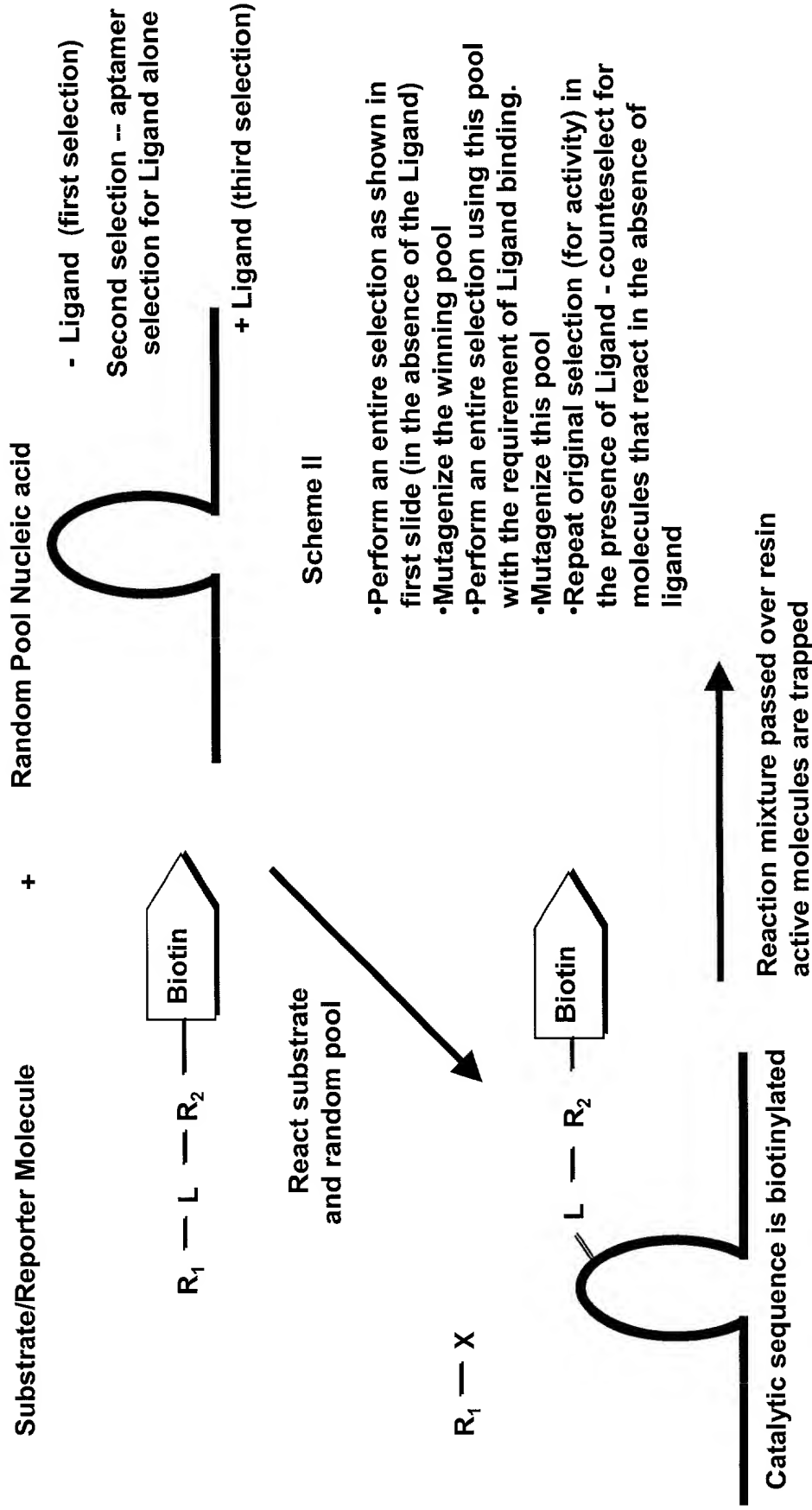
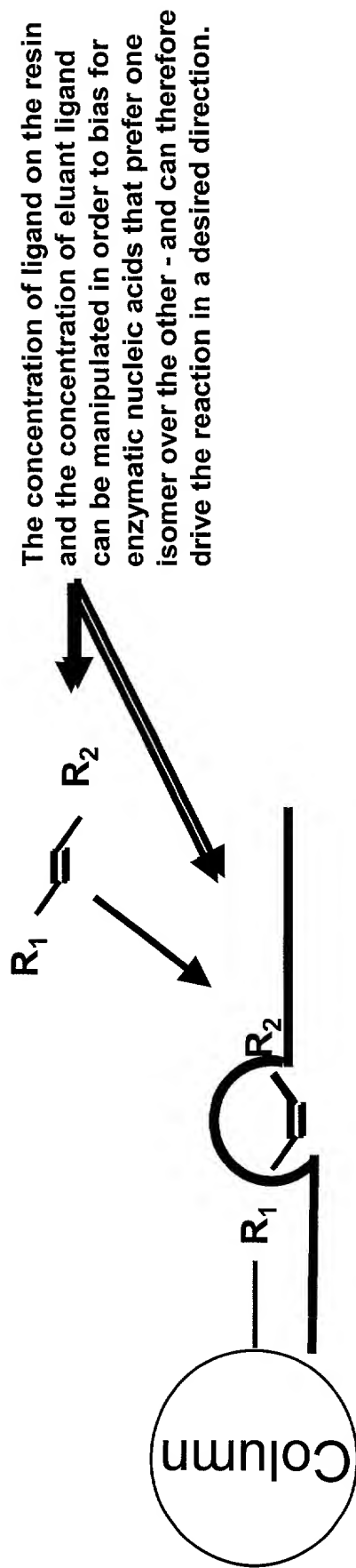


Figure 18b: Isomerase Nucleic Acid Sensor Molecule - Selection Scheme



E.g. Selection for Cis-isomer at 100 μM - yield $\text{cis}K_d = 100 \mu\text{M}$
 Elute with Trans-isomer at 0.1 μM - yield $\text{trans}K_d = 0.1 \mu\text{M}$

Isolate catalysts for the reaction below



Figure 18c: Isomerase Nucleic Acid Sensor Molecule - Ligand dependent

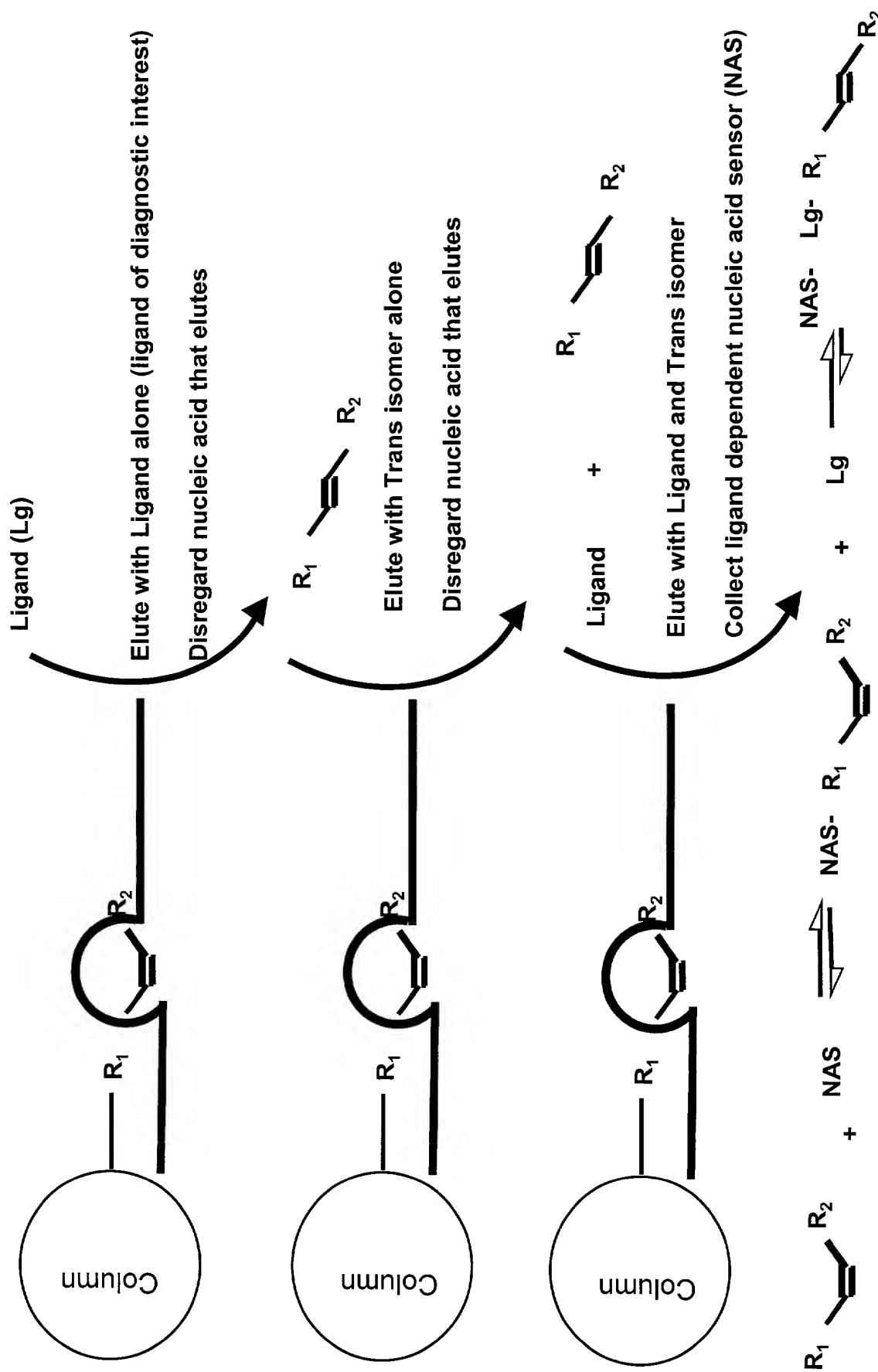
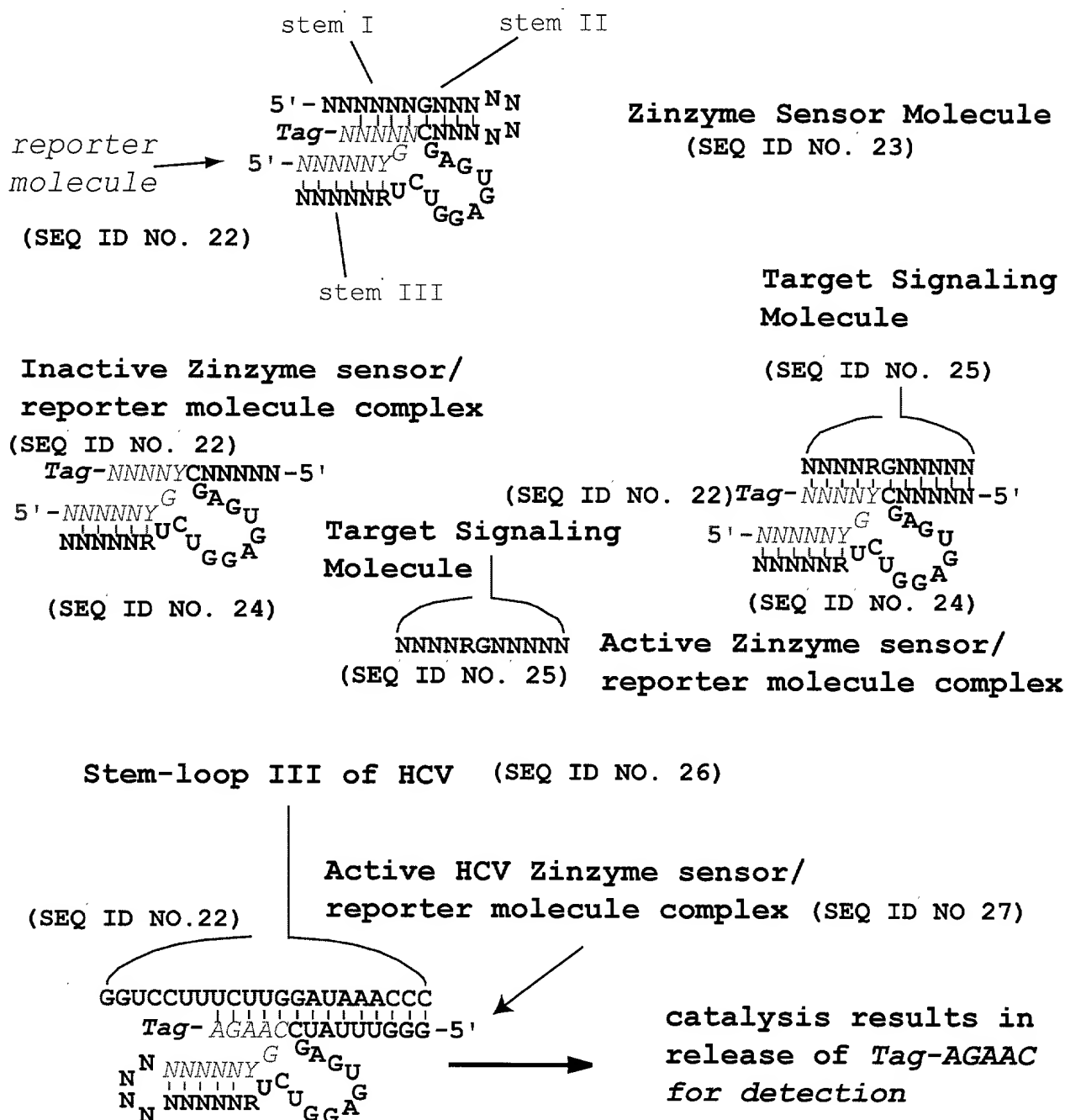


Figure 19: Zinzyme Sensor Molecule for detection of Nucleic Acid



Zinzyme sensor can be attached to solid support/surface, for example at the 5'-end

105011-0912660

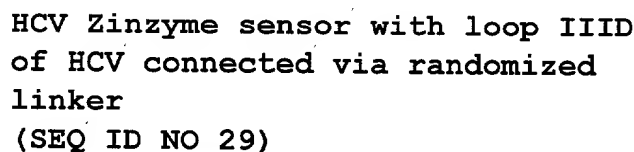
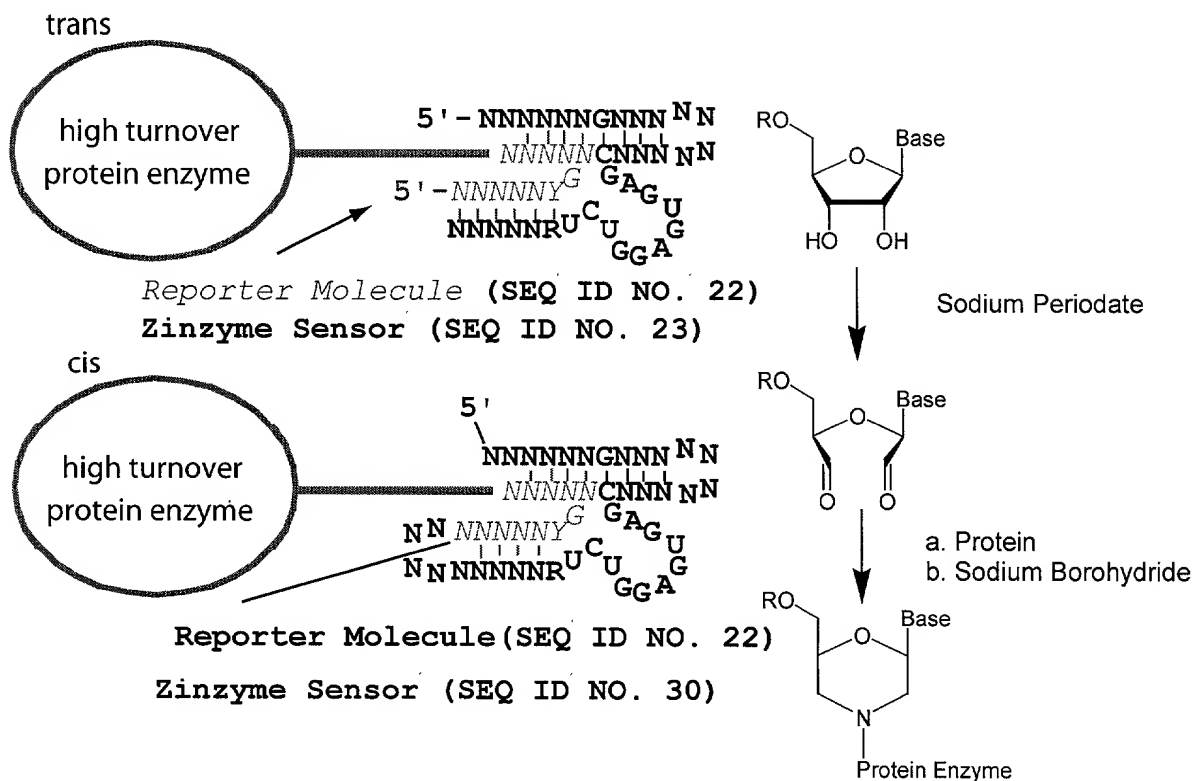


Figure 21: Zinzyme Sensor Molecule with protein enzyme reporter



R is oligonucleotide.

Protein can be attached via amino linker.

Alternately, R is phosphoramidite moiety for incorporation at 5'-end of oligonucleotide.

High turnover protein enzyme is, for example, Luciferase, Horseradish peroxidase, beta-galactosidase, alkaline phosphatase.

Amplification of signal via use of protein enzyme conjugate

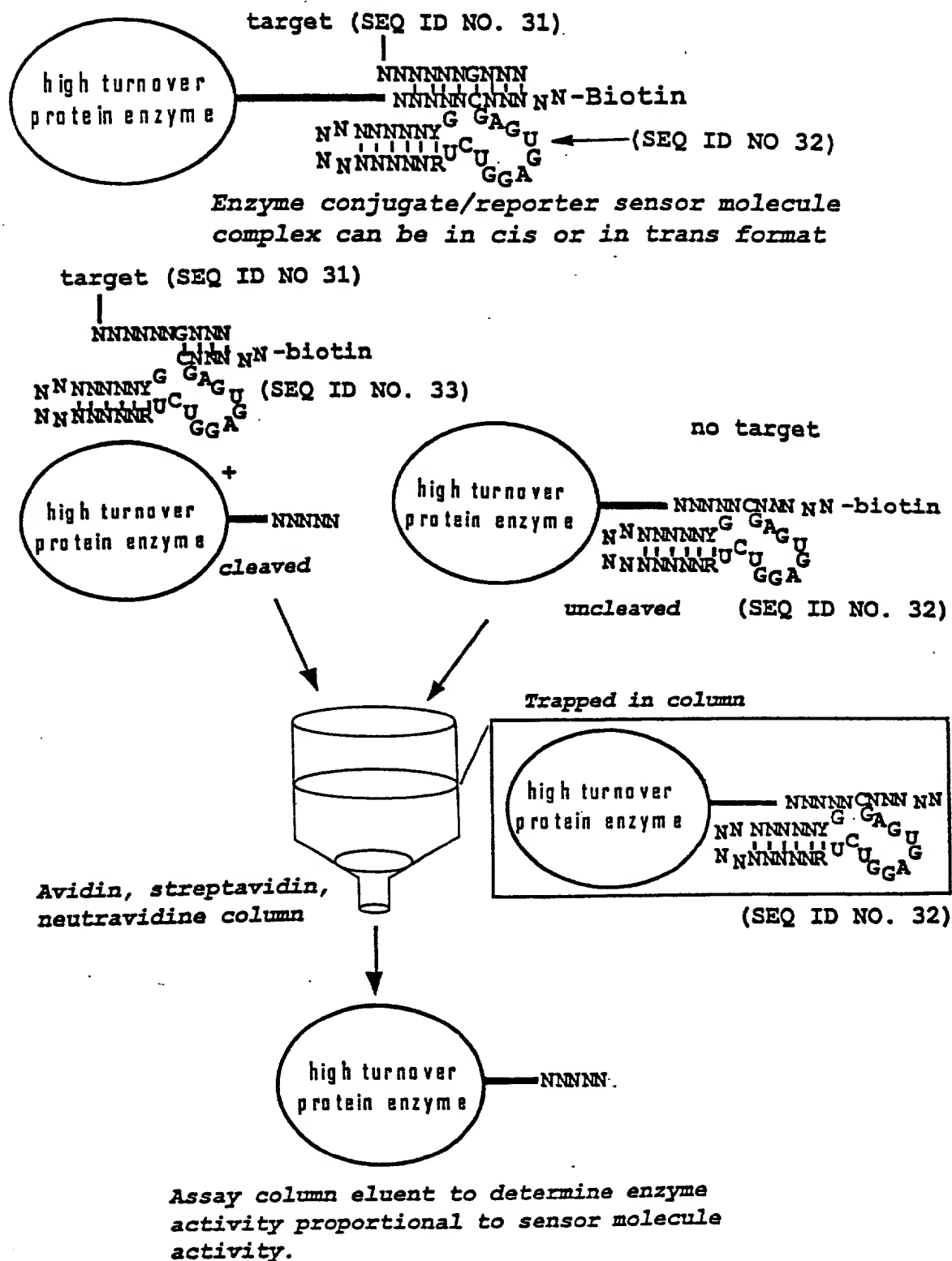
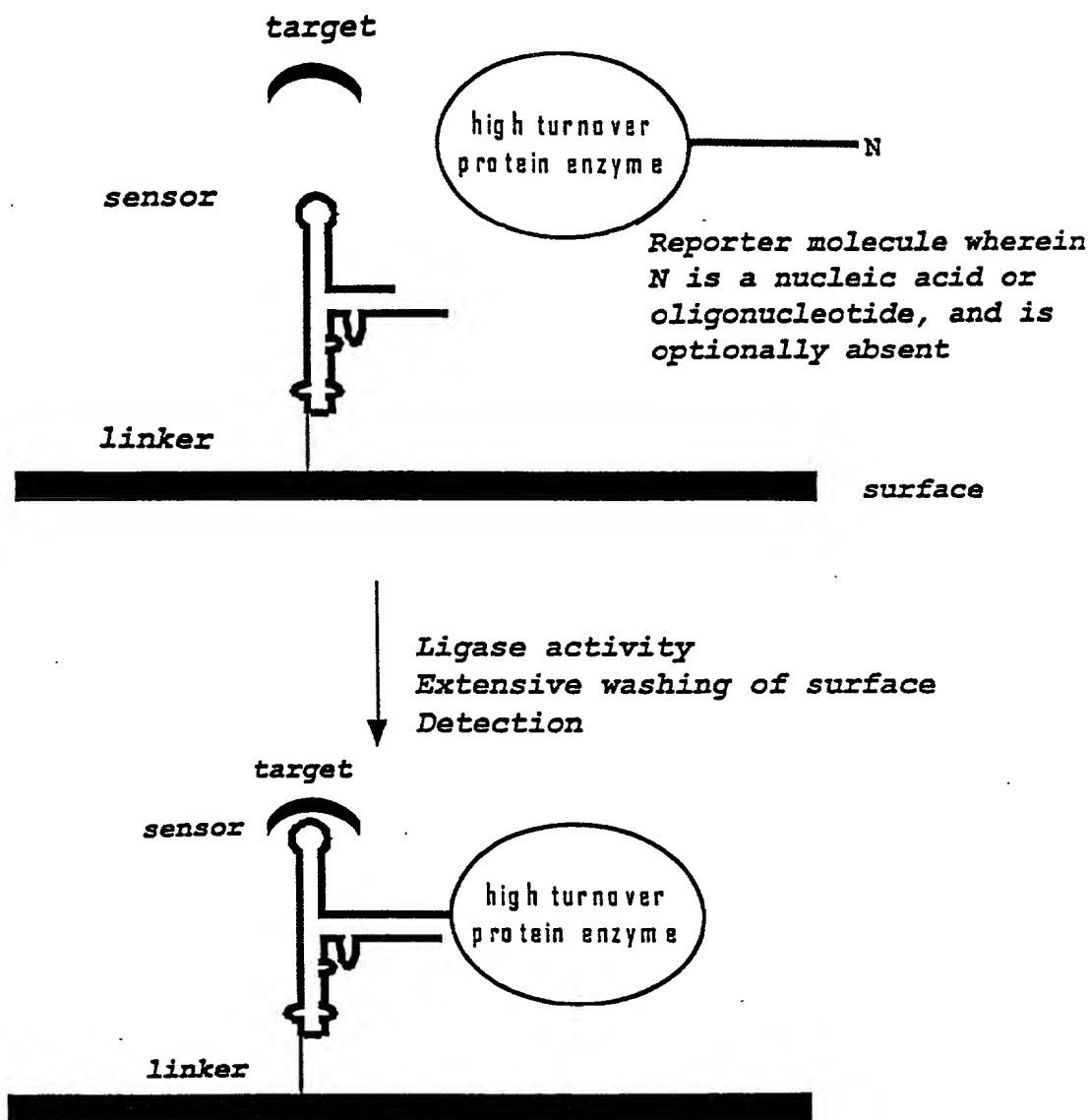


FIG. 22

Ligase Sensor Molecule with enzymatic reporter



Alternatively, a fluorescent or chemiluminescent based reporter molecule is used.

FIG. 23

Figure 24: Selection of Nucleic Acid Sensor Molecules with Ligase Activity

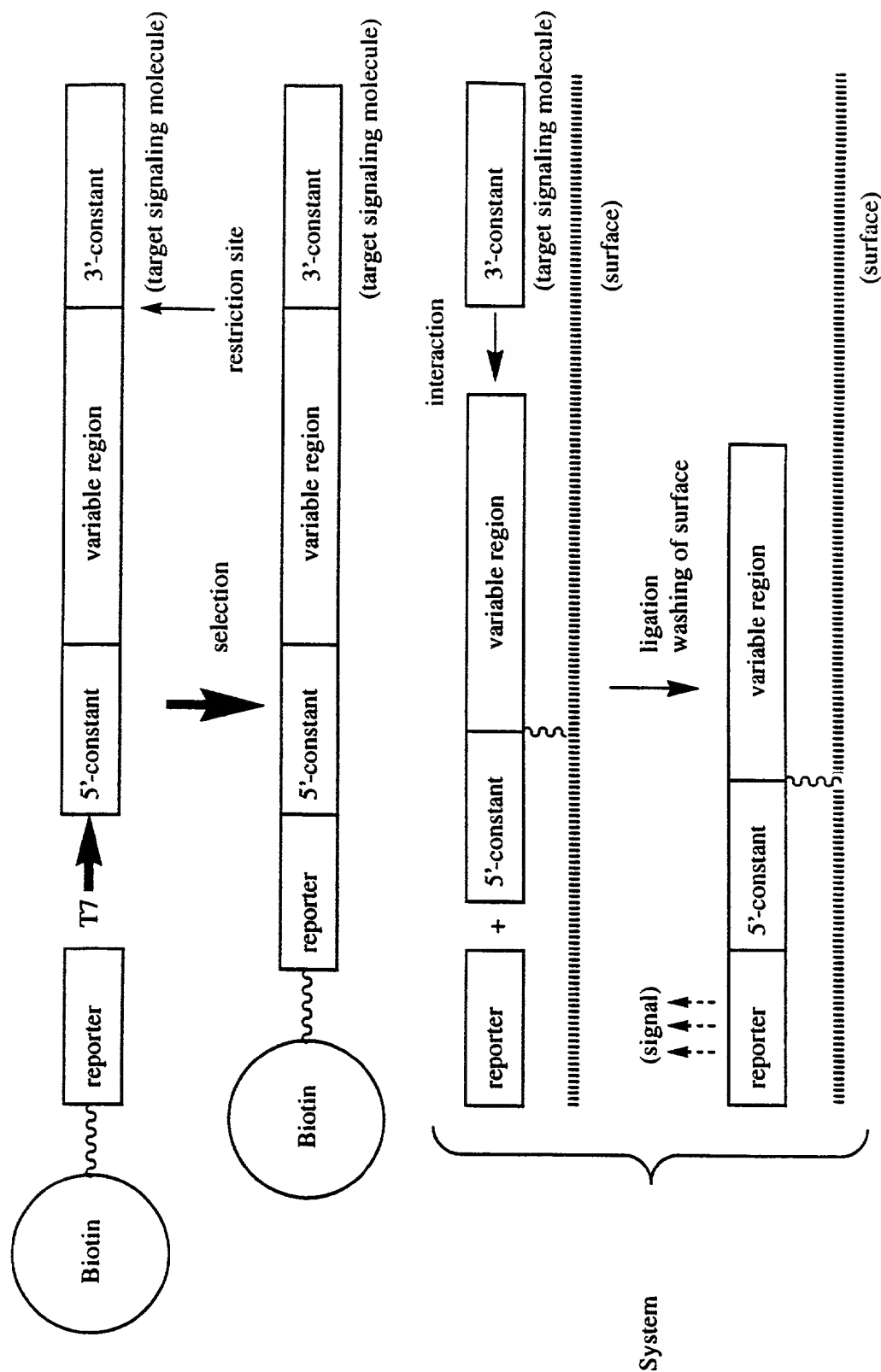


Figure 25: Nucleic Acid Sensor Molecule-Based Electric Circuit

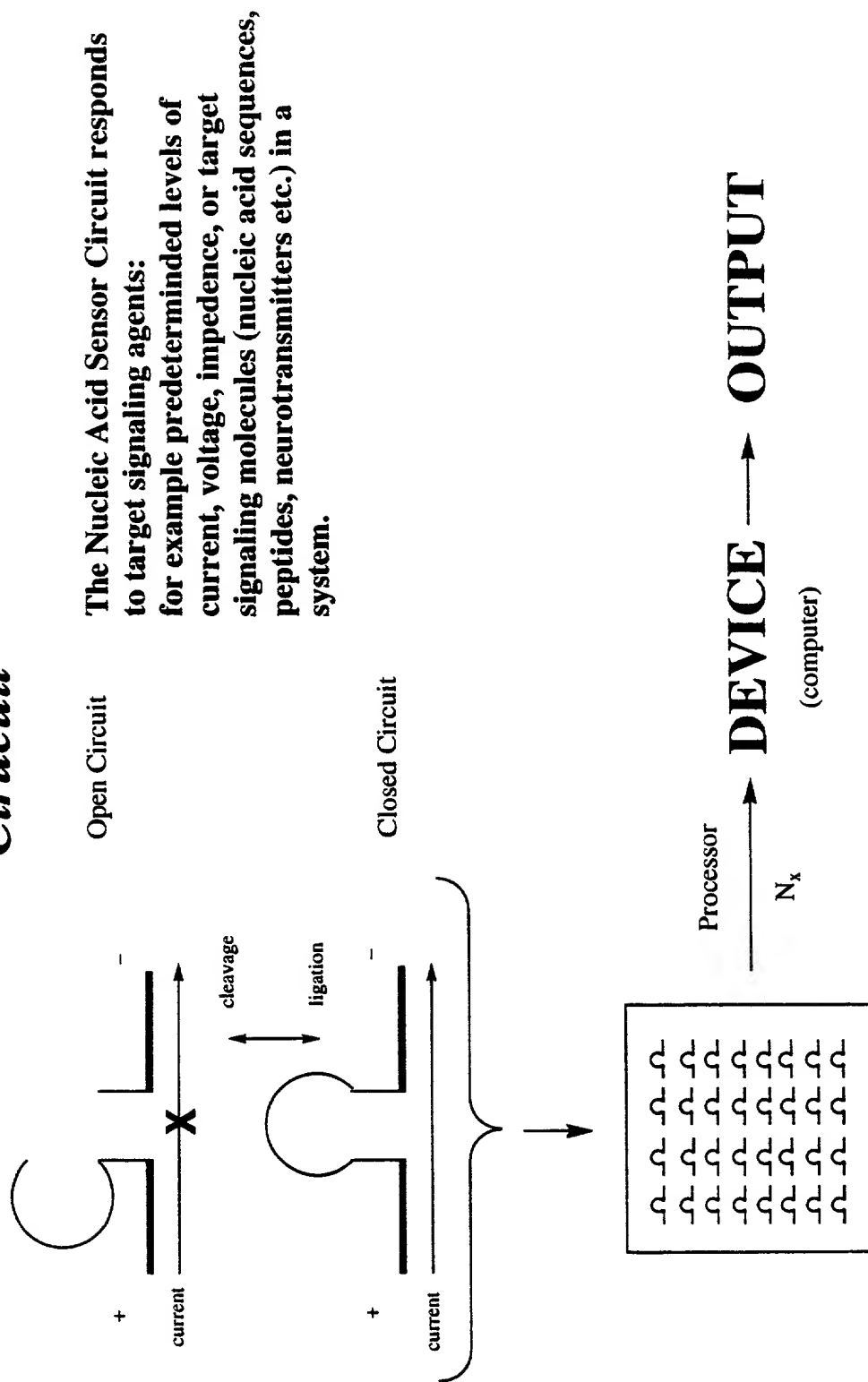
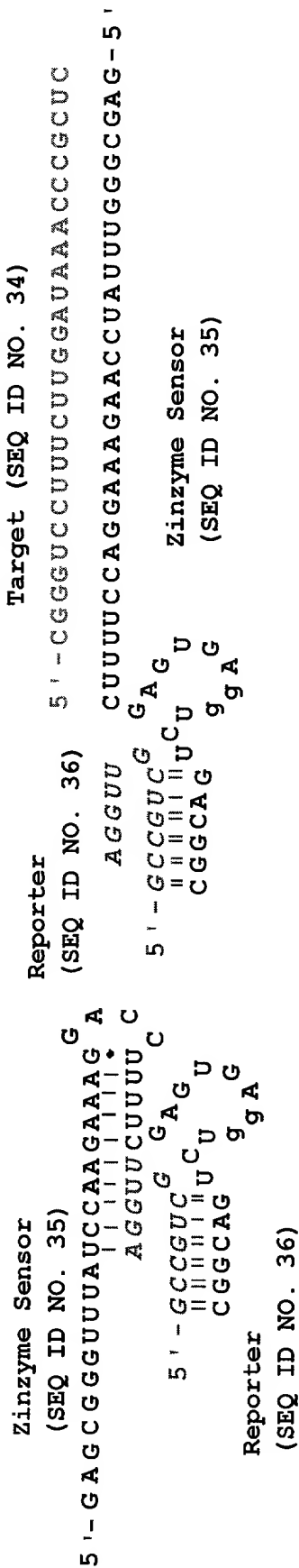


Figure 26: Target Inactivation of Zinzyme Sensor Molecule



ACTIVE \longleftrightarrow TARGET INACTIVATED

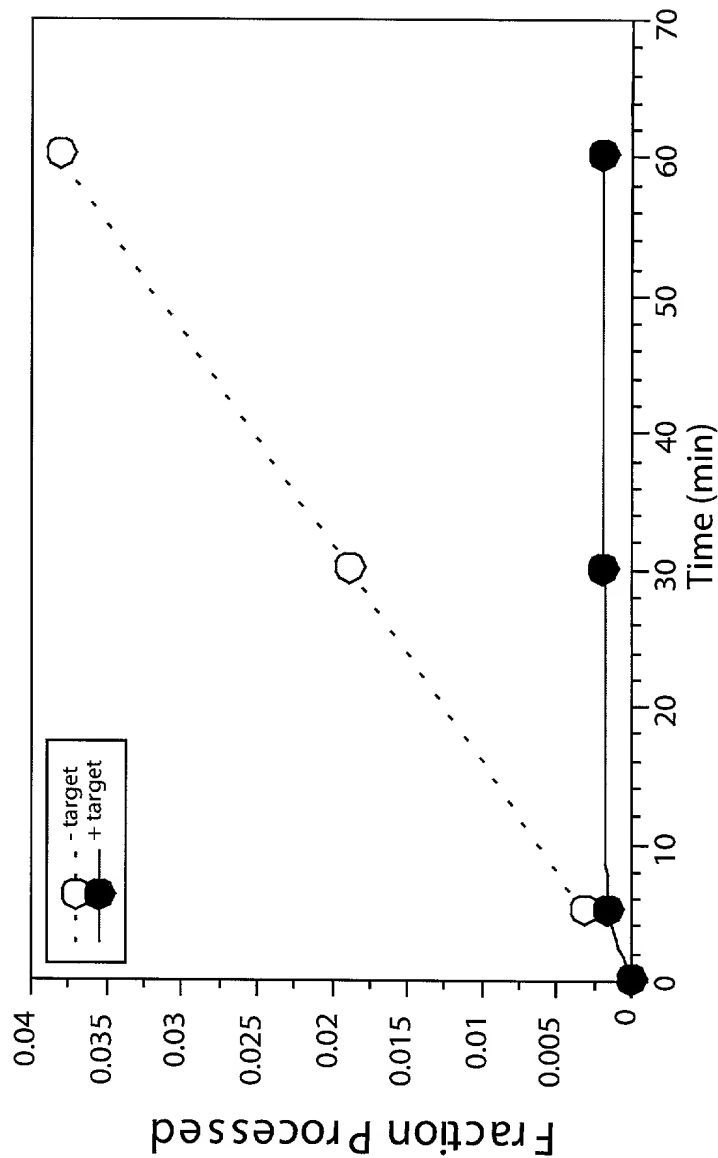


Figure 27: Target Activation of Zinzyme Sensor Molecule

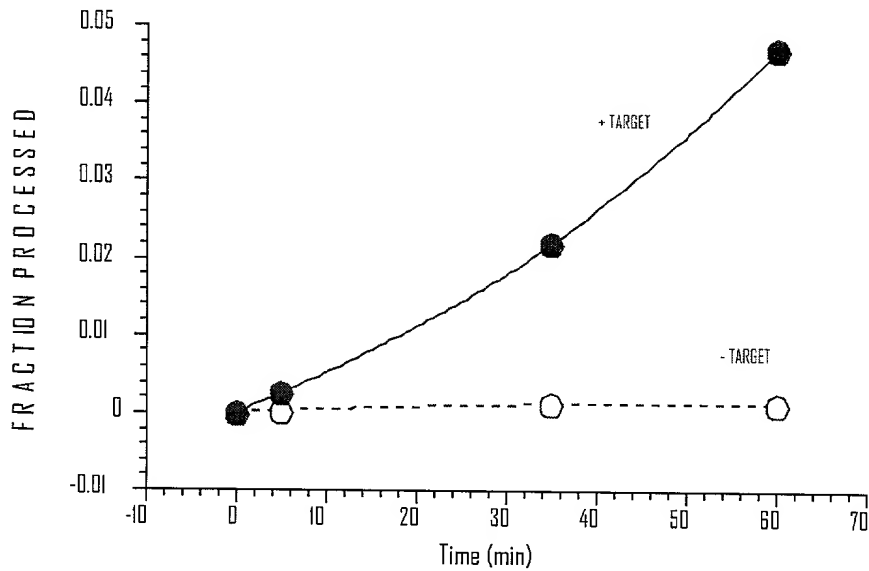
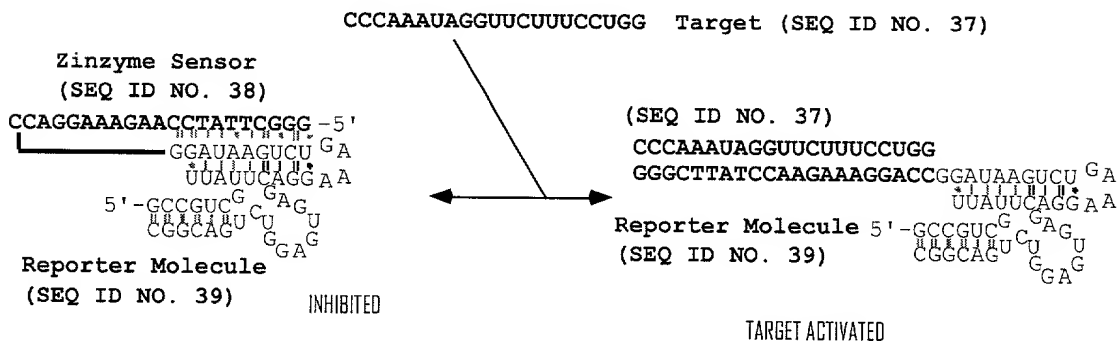
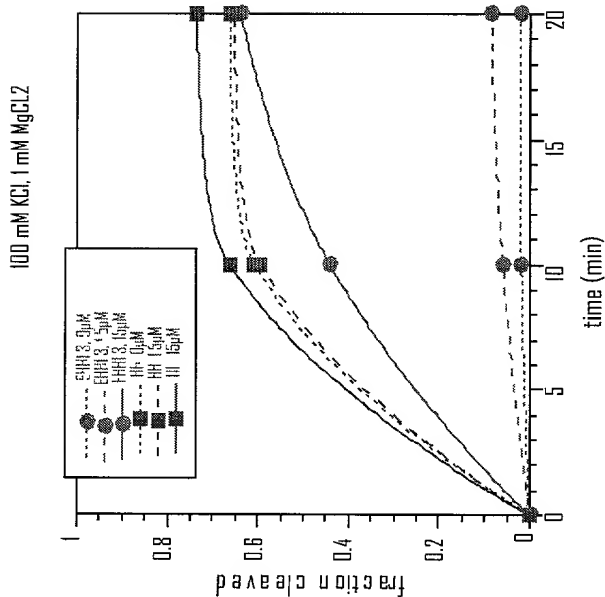
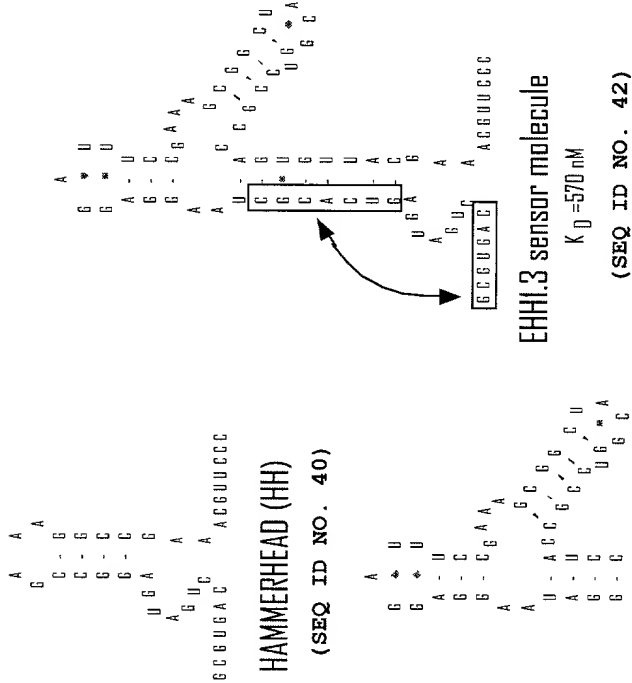


Figure 28: Erk modulated Nucleic Acid Sensor Molecule



Half-Zincyme
(SEQ ID NO. 43)

Cleavage site

5'-GGUCCUUCUUGGAUAAACCC-3'

5'-GGUCCUUCUUGGAUAAACCC-3'

Target Signaling Molecule (SEQ ID NO. 26)

Active

Inactive

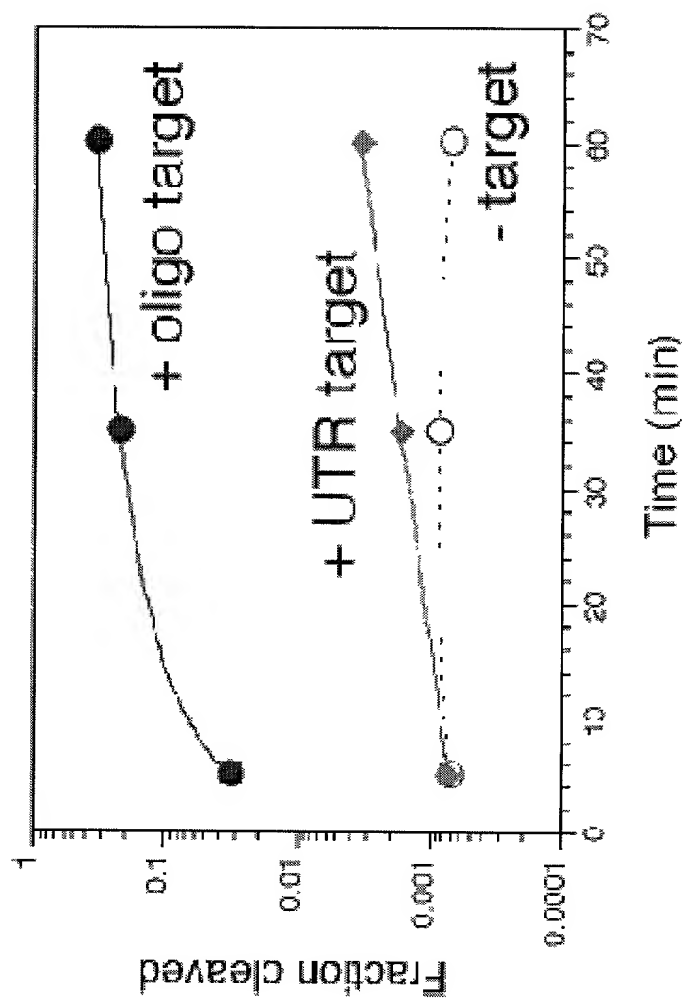


Figure 30

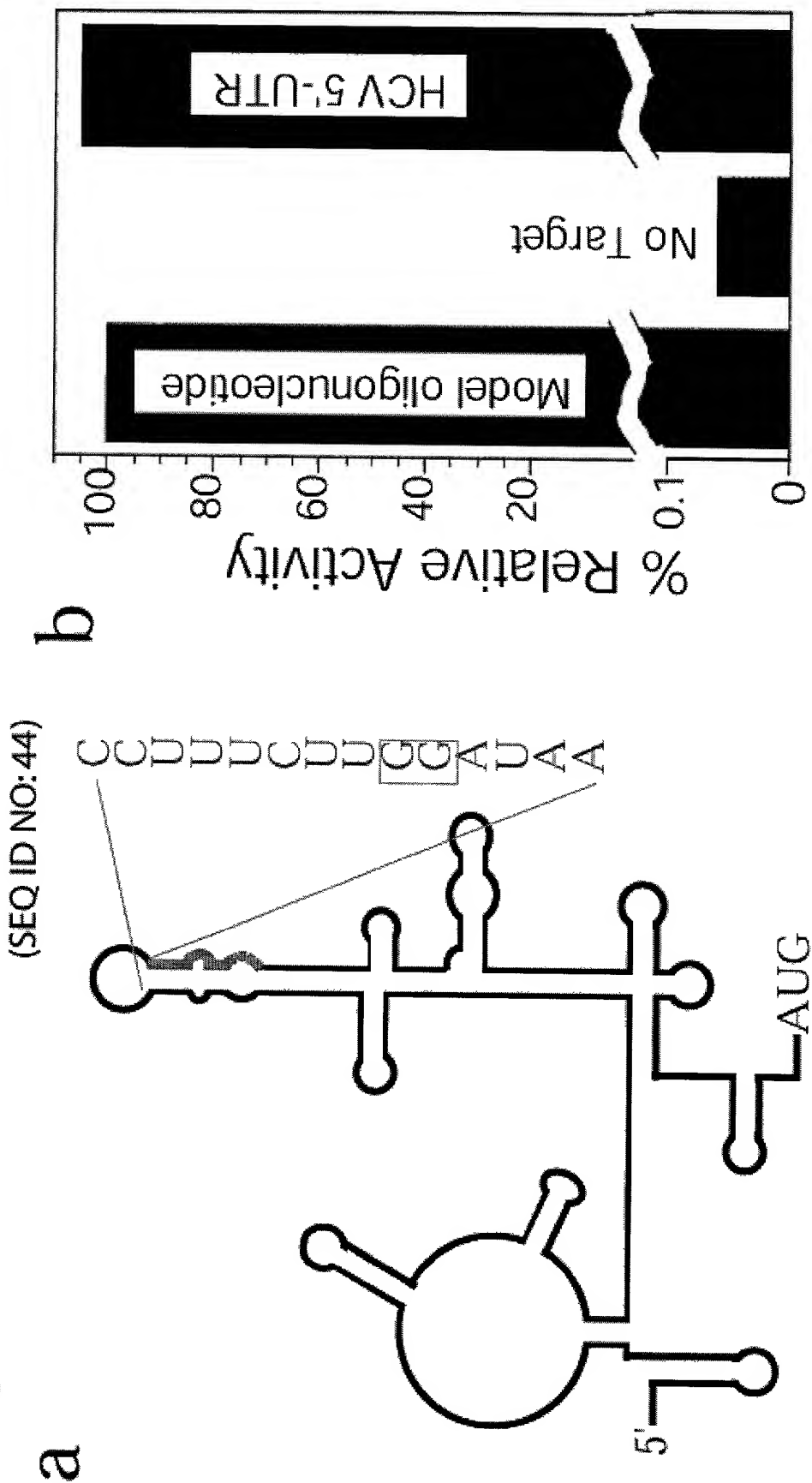


Figure 31

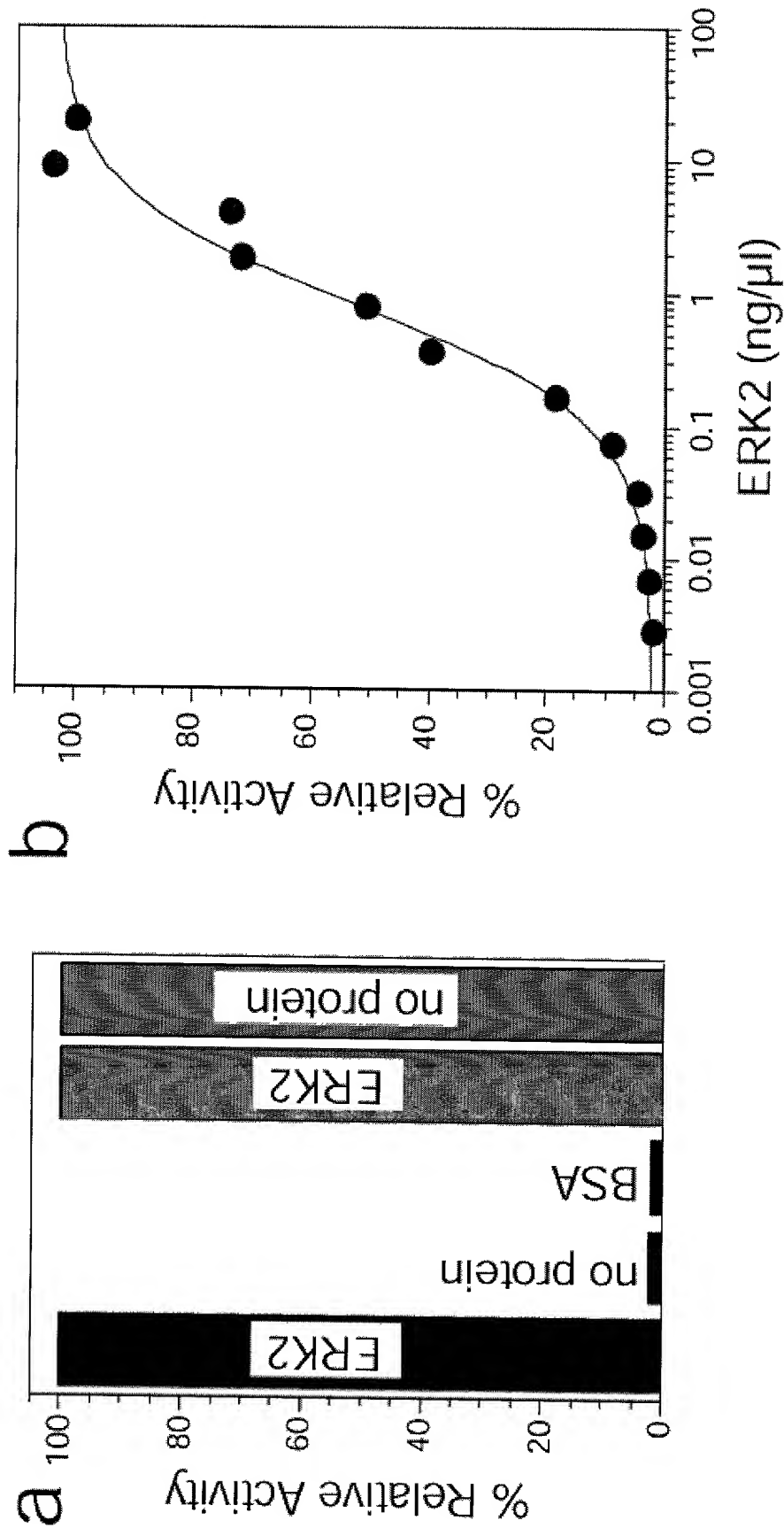


Figure 32

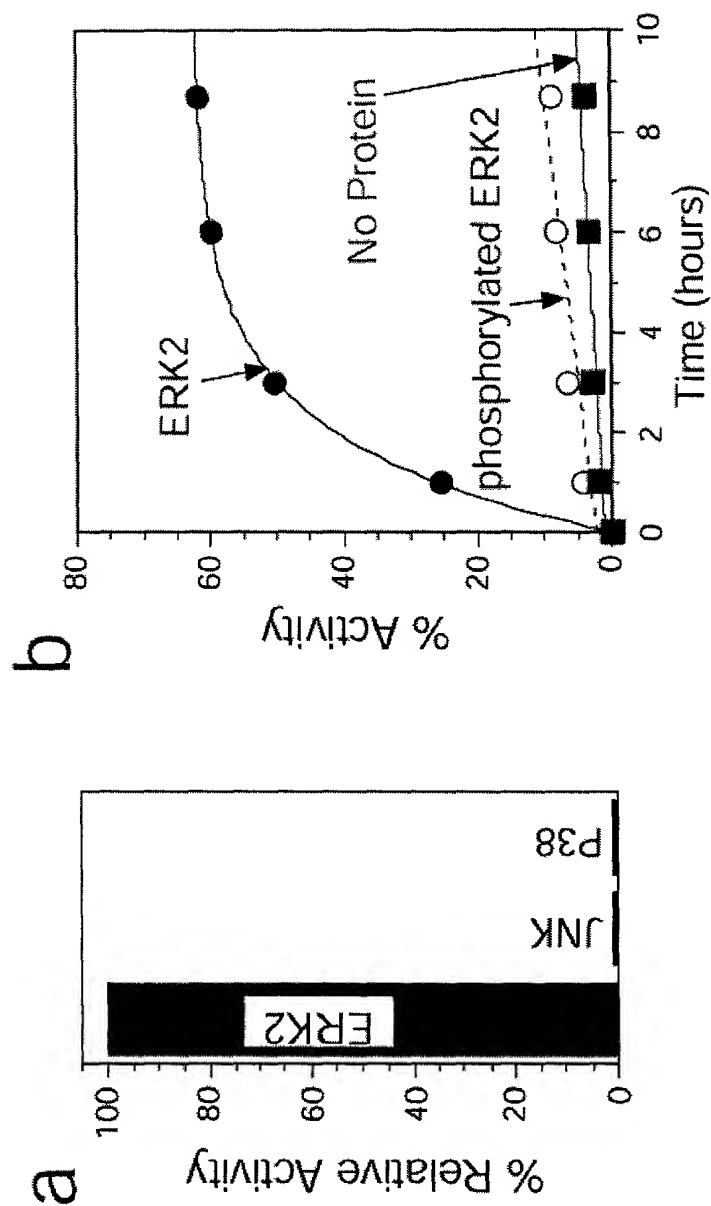
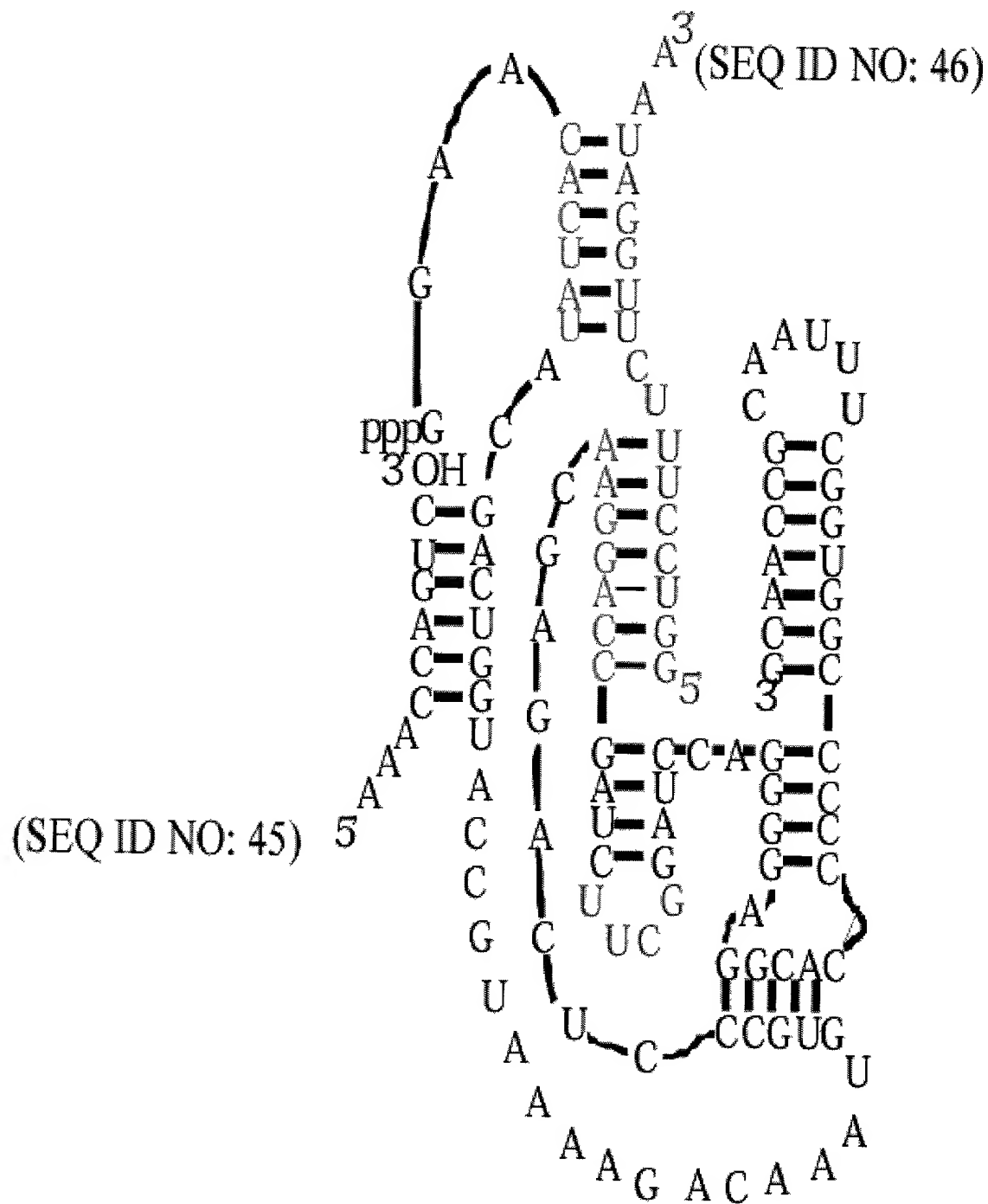
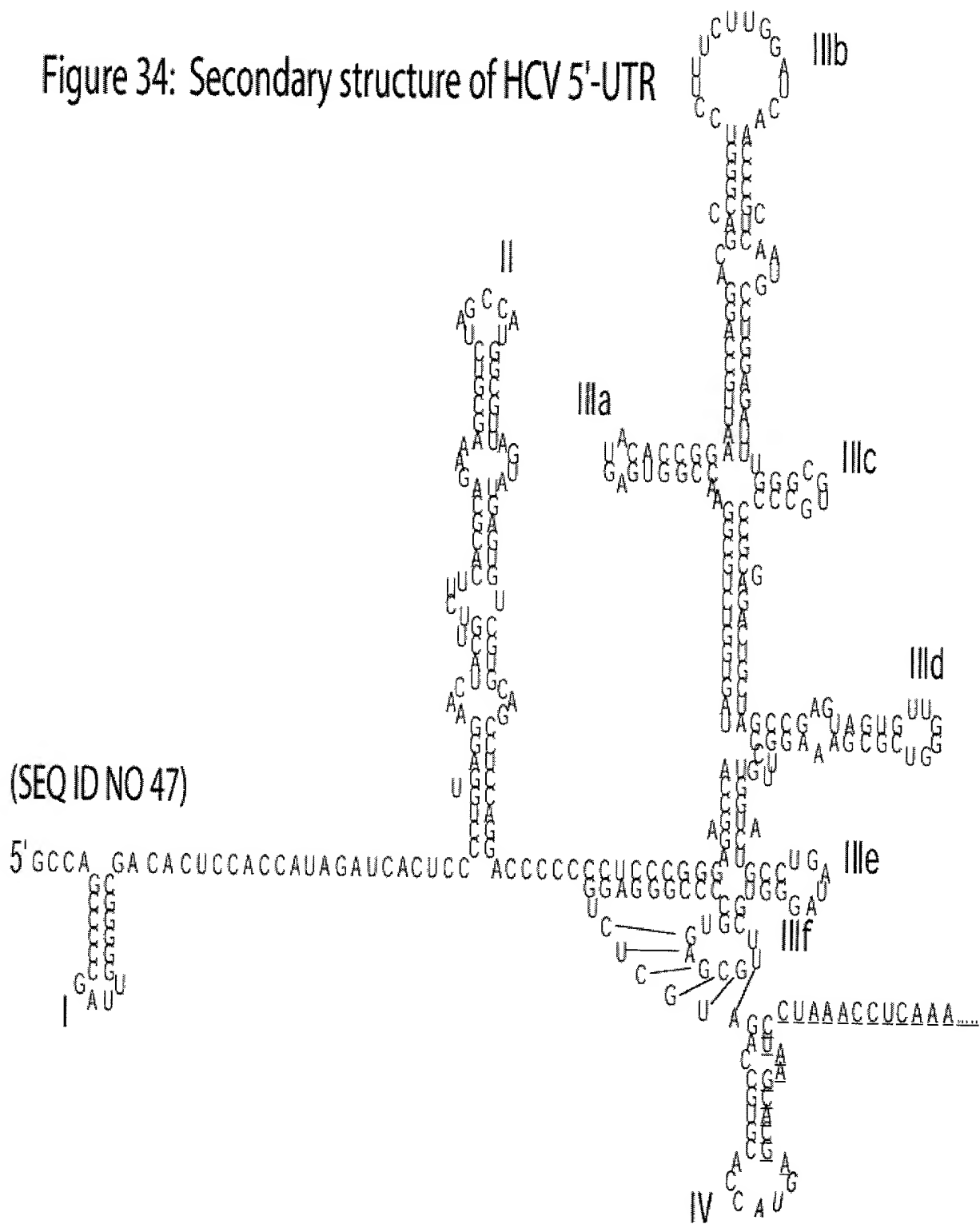


Figure 33: Halfzyme Ligase



105077-09726550

(SEQ ID NO 47)



SEQ ID NO:

58

Figure 36: Single Nucleotide Polymorphism (SNP) Detection

